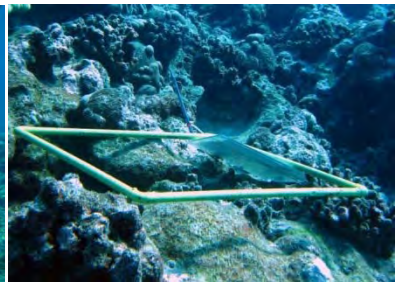
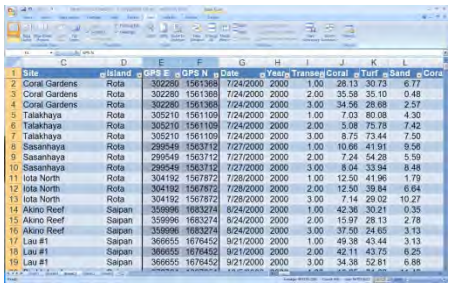
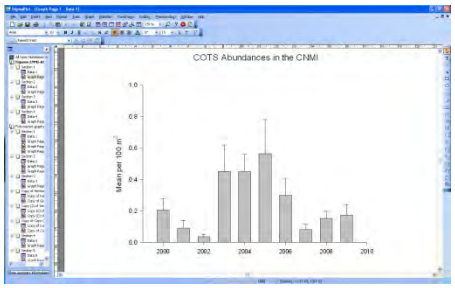
Improving Local Capacity for Coral Reef Monitoring Data Interpretation

A guidebook with step-by-step exercises to improve local capacity for data collection, storage, handling, visualization, and analysis throughout Micronesia.

Dr. Peter Houk

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Introduction:

Statistically-sound science is required to assess the status of regional and local management efforts ranging from community-based marine protected areas to expansive regional networks defined by the Micronesian Challenge. Despite having common goals of protecting their resources for future generations, jurisdictions throughout Micronesia strongly differ in their approach used to monitor coral reefs, and thus, in the information that is available for managers to act upon. This is, in part, due to unequal funding and capacity distributed throughout the region. As of 2009, monitoring throughout Micronesia ranged from reef-check surveys conducted by governmental and recreational divers in Kosrae, to seven-year programs supporting multiple trained biologists in the Commonwealth of the Northern Mariana Islands and Palau. Accordingly the questions being answered, statistical power to detect change, and the precision of the data differ considerably (Houk and van Woesik, 2006; Houk 2009; Waddell and Clarke, 2008).

Recently, the 5 political jurisdictions of Micronesia have begun to address these issues, under the framework of the Micronesia Challenge. In June 2008, the newly formed MC Measures Working Group identified the need to develop an appropriate framework to assist monitoring programs in each of the jurisdictions to track their progress both locally and regionally in effectively managing their resources for sustainable use. Spawning from the goals set forth by the MC Measures Working Group collaborations between the Pacific Marine Resources Institute (PMRI), Dr. Peter Houk, and jurisdictional monitoring programs were conducted to evaluate the status of existing datasets in 2009. This effort initiated positive, continued collaborations for enhanced scientific oversight of monitoring activities with numerous regional partners and scientists.

Building upon a scientific foundation to match management goals with monitoring activities, key recommendations were made to initiate a standardized monitoring approach for the MC, and beyond (Houk 2009). These designs and methods were tested in each jurisdiction, and shown to address many pressing management concerns with adequate statistical considerations. Since 2009, data has been collected using the updated techniques, and now exists. However, these data are not being thoroughly examined and reported on because, generally, the scientific expertise needed to digest the collected data for management has not yet been well developed within local programs. This forms the basis for the development of this guidebook.

Here, we present a step-by-step data analyses and graphing guidebook that was produced by PMRI and funded by NOAA Pacific Islands Marine Protected Area Community program. This guidebook was developed using regional data collected during FY 09 collaborations between PMRI and FSM/RMI coral monitoring programs. The goal is to bring key users of datasets from each FSM and RMI jurisdiction together for a “hands on” workshop to evaluate their data, and learn how to efficiently visualize and, when appropriate, test for significance. The guidebook represents a framework for this process. The guidebook was produced using four major software platforms: Microsoft Excel, Access, PRIMER-E, and Sigma Plot. These will all be needed to use this guidebook. Please provide constructive comments regarding the guidebook to PMRI through their website (www.pacmares.com).

Section 1 – Database generation, manipulation, and query investigation

Exercise 1 – Establishing a database

The initial establishment of a database can often seem like a daunting task for us. Consider that a wealth of information is typically required, or at least desired, and all of that information resides with multiple people or agencies. However, Micronesia's coral monitoring programs are often limited in personnel and capacity, so learning to do the best possible job with the resources at hand is a logical outcome. There is no one right way to format a database, several different approaches can lead to similar outcomes. However, some basic rules do apply. When deciding upon how to format a database the first question that one should ask is what is being measured and what is the unit of replication. Two examples below will show different approaches.

Example 1 – Macroinvertebrate data collection from Chuuk Rapid Ecological Surveys

Rapid ecological assessments were conducted in Chuuk during August-September 2008. In this example we will build a desirable Microsoft Excel database to store the quantitative macroinvertebrate estimates that were made.

1. Open Excel
2. Select and **open** the file "**Chuuk-REA-invert-database**" from the example file directory on your computer.
3. Click on the first sheet "**Brainstorm metadata fields**" to understand the nature of data collection.

Data were collected from 8 islands among a variety of reef types and wave exposures. At each site surveyed, two depths were examined for macroinvertebrate abundances along 5 transects. A transect consisted of a 5-minute timed swim. The observer recorded all conspicuous macroinvertebrates that were observed. No size data were collected, just counts. From the "Brainstorm metadata fields" sheet you can get an idea of the sampling scheme. This is a logical first step in creating a database, to generate a 'brainstorm' or relevant metadata fields sheet that breaks down how sampling was conducted.

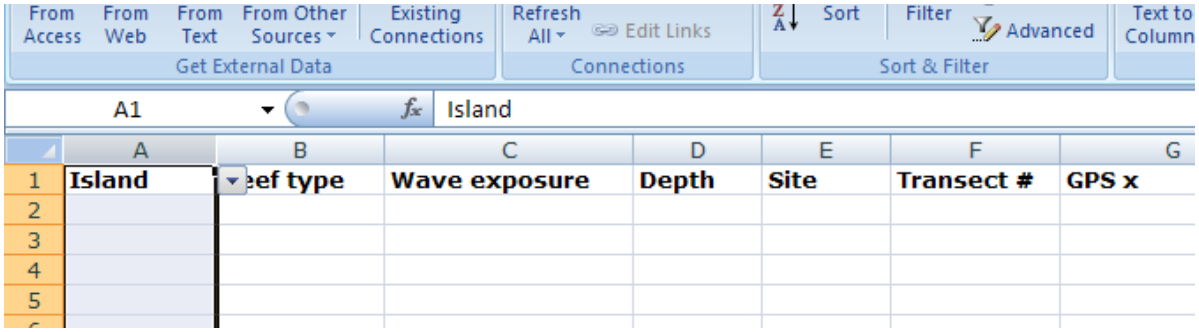
4. Click on the next tab "**Metadata**".

Here a list of all sites surveyed was populated while surveys were being conducted. Location information exists as well as site characteristics. Armed with this information laid out in this manner, were ready to begin building our database.

5. Click on the next tab "***Database-build***".

Notice that the metadata headings from the earlier sheets are copied over already. We need to populate them, and reduce the chance of data entry error.

6. Click on the "***Data***" tab on the main menu of excel – up top.
7. Click on the entire "**A**" column above the word "**Island**" – see below



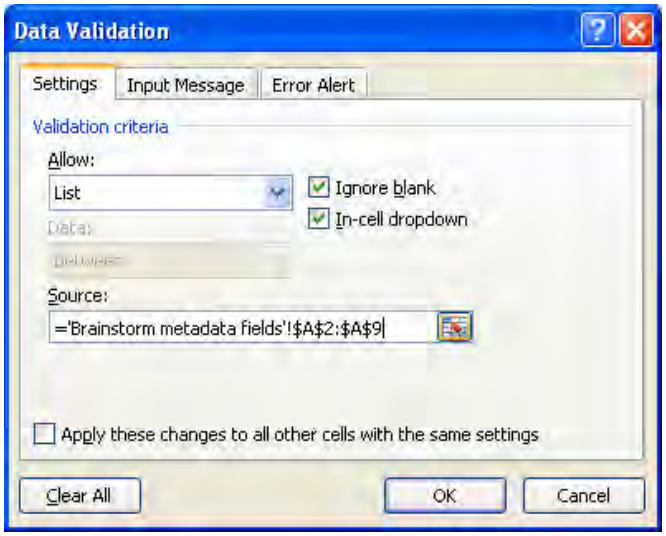
8. Now under the excel sub-menu "**Data Tools**" click on "**Data Validation**"

a) The menu below should pop-up. Click on the "**Settings"** tab.

b) Then for validation criteria, scroll down until you find the "**List**".

9. Now you should see a "**Source**" field open up, where you want to provide the selective criteria for entering data into these cells (i.e., the island names where data was collected from).

a) Type in exactly what is seen below.

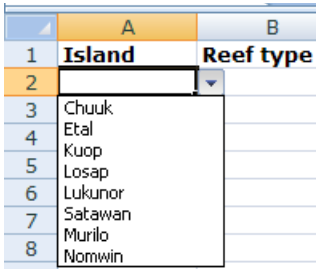


10. Click OK

This code logically refers Excel to the sheet named "Brainstorm metadata fields", and says that all possible islands where data were collected from are located in cells A2:A9. Verify that for yourself. *(**Note**: When doing this, it is very desirable to have scratch paper for taking informal notes to assist you with entering source codes and functions into excel.)*

11. Click in cell **A2** and notice there is a dropdown arrow on the right hand side.

a) Click the **drop down arrow** and notice the list of islands appears where data were collected from.



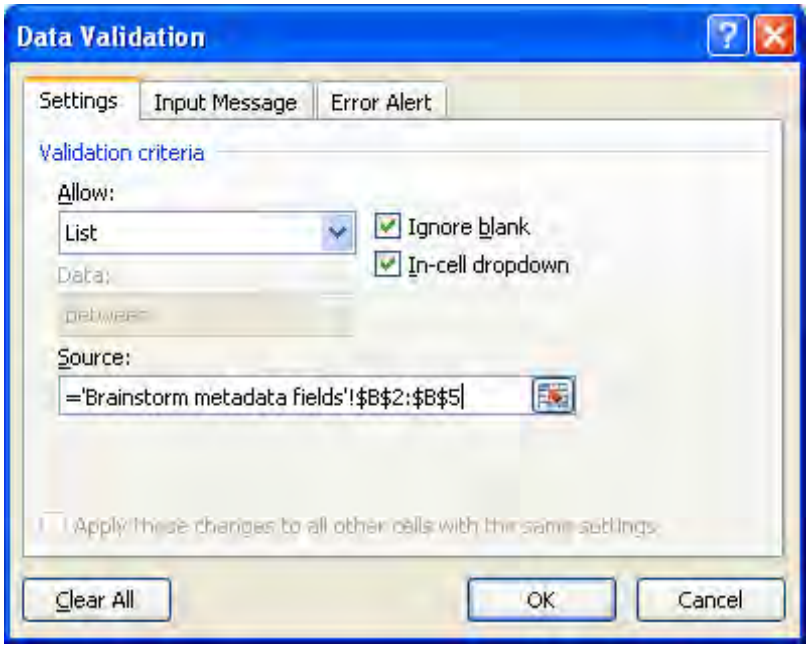
b) Choose any island name for now.

c) Click in cell **A3**, do the same. **Populate** cells down to **A10**.

12. Do the same for the next column "**Reef type**"

a) Click on the column "**B**" on top of "**Reef type**"

b) Type the below code into the empty "**Source**" box.



13. Again, verify yourself why cells B2:B5 are chosen by examining the "**Brainstorm metadata fields**"
Populate cells **B2:B10** with values of your choosing.

	A	B	C	D	E	F	G	H
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel						
3	Chuuk	Inner						
4	Chuuk	Inner barrier						
5	Etal	Outer barrier						
6	Losap	Outer barrier						
7	Losap	Channel						
8	Murilo	Inner barrier						
9	Etal	Channel						
10	Chuuk	Inner						
11		Channel						
12		Inner						
13		Inner barrier						
		Outer barrier						

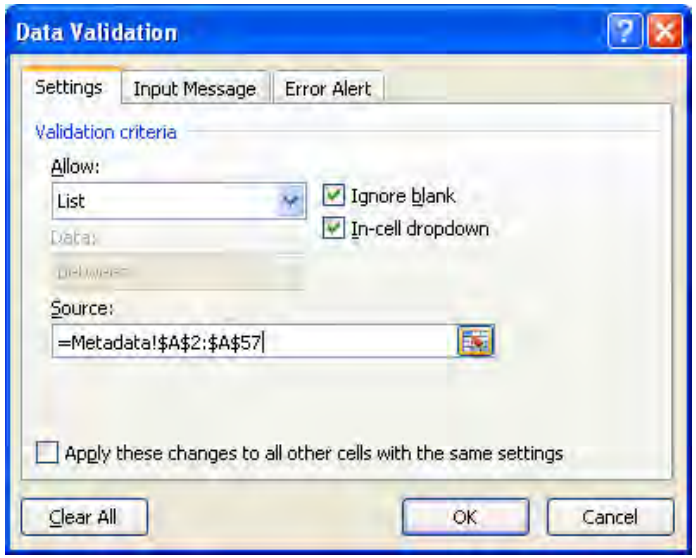
14. Do the same for the next column "**Wave exposure**" and "**Depth**"

a) Fill in columns with values of your choosing.

D10 | fx | 10m

	A	B	C	D	E	F	G	H
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel	Low	3m				
3	Chuuk	Inner	Moderate	3m				
4	Chuuk	Inner barrier	Low	3m				
5	Etal	Outer barrier	Sheltered	3m				
6	Losap	Outer barrier	Moderate	3m				
7	Losap	Channel	High	10m				
8	Murilo	Inner barrier	Low	10m				
9	Etal	Channel	Moderate	10m				
10	Chuuk	Inner	Moderate	10m				
11				3m				
12				10m				

15. Now, do the same for the "**Site**" column, but change the source as follows



Note that we reference a different sheet, the "**Metadata**" sheet now.

a) Click on that sheet and verify that ***A2:A57*** were selected.

b) Populate the database with values of your choosing.

16. Now for the next column "**Transect** #:" we can again do the list function, or we can simply write the numbers 1 – 5. You choose and populate the cells.

F2 | 1

	A	B	C	D	E	F	G	H
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel	Low	3m	C-1	1		
3	Chuuk	Inner	Moderate	3m	C-11	1		
4	Chuuk	Inner barrier	Low	3m	C-13	2		
5	Etal	Outer barrier	Sheltered	3m	C-14	2		
6	Losap	Outer barrier	Moderate	3m	C-13	3		
7	Losap	Channel	High	10m	C-14	3		
8	Murilo	Inner barrier	Low	10m	C-16	4		
9	Etal	Channel	Moderate	10m	M-4	4		
10	Chuuk	Inner	Moderate	10m	M-9	5		
11								

Now for the fields "***GPS X***" and "***GPS Y***" we will use a "***lookup***" function because there are too many numbers in the GPS coordinates to try and process through a dropdown list.

17. Click on cell ***G2***

a) Type the following in the function toolbar:

SUM | =LOOKUP(E2,Metadata!$A$2:$A$57,Metadata!$C$2:$C$57)

	A	B	C	D	E	F	G	
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel	Low	3m	C-1	1	!$C$2:$C$57)	
3	Chuuk	Inner	Moderate	3m	C-11	1		
4	Chuuk	Inner barrier	Low	3m	C-13	2		
5	Etal	Outer barrier	Sheltered	3m	C-14	2		

The "lookup" function first asks for the reference cell value upon which the lookup will occur, in this case it is cell E2, or the "site". Next, you have to provide a list of all possible sites for excel to look up, in this case the list is found on the "Metadata" sheet, columns B and C are the X and Y coordinates (i.e., lat and long) for each site. Now, you do this for the GPS Y, or latitude, coordinate.

Note: It is important to note that the "$" in the cell formula means for Excel to keep the exact cells when conducting the functions. Without them, the references for the lookup values would change when we cut and paste into cells below to automatically populate our database.

18. Get a scratch paper out, **click** on the "***Metadata***" sheet, and note which cells that contain ***GPS Y*** coordinates you are interested in, and the site names associated.

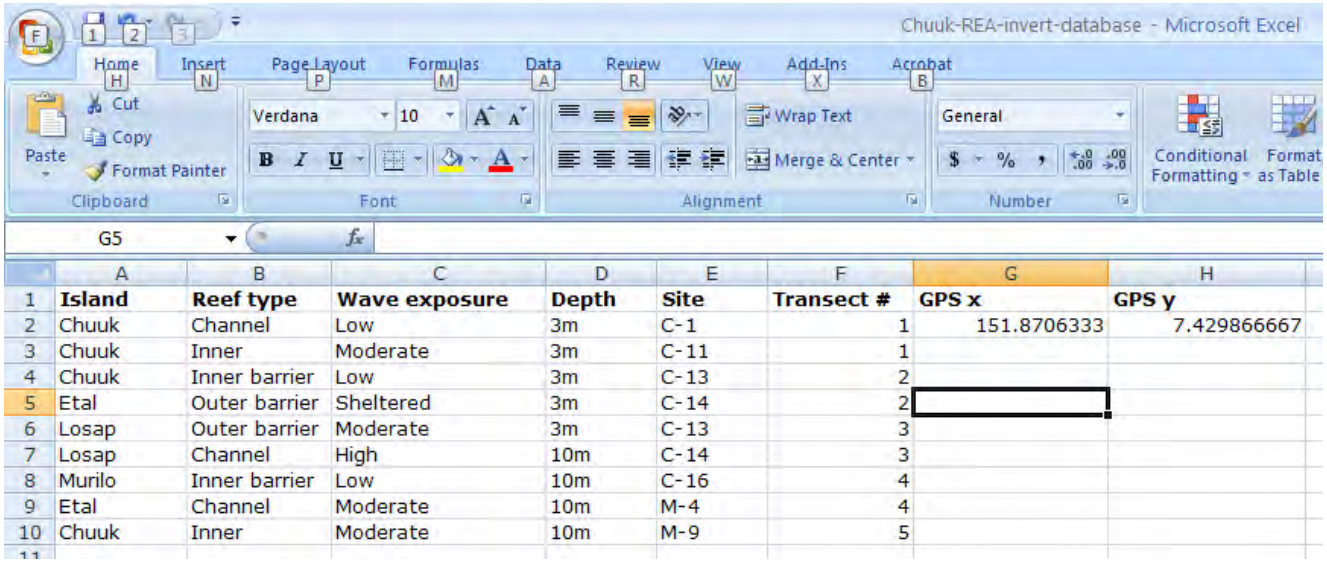
a) The relevant information to write are the site names that will be looked up (***A2:A57***, on the "***Metadata sheet***"), and the valued you want inserted, ***GPS Y*** (***B2:B57***).

19. Go back to our "**Database-build**" sheet and **highlight** the first cell you want to populate with the "**lookup**" function for **GPS Y**, or latitude. This is cell ***H2***.

a) Once highlighted, enter the appropriate formula: ***=lookup(E2, Metadata!$A$2:$A$57, Metadata!$B$2:$B$57)***

Note: we do not want and $ near the E2 because that is dynamic, and we want to drag our formula to autofill the cells below. However, we want $ to appear for all lookup list values on the "Metadata" sheet. These will never change.

b) Your database should now look like below.



	A	B	C	D	E	F	G	H
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel	Low	3m	C-1	1	151.8706333	7.429866667
3	Chuuk	Inner	Moderate	3m	C-11	1		
4	Chuuk	Inner barrier	Low	3m	C-13	2		
5	Etal	Outer barrier	Sheltered	3m	C-14	2		
6	Losap	Outer barrier	Moderate	3m	C-13	3		
7	Losap	Channel	High	10m	C-14	3		
8	Murilo	Inner barrier	Low	10m	C-16	4		
9	Etal	Channel	Moderate	10m	M-4	4		
10	Chuuk	Inner	Moderate	10m	M-9	5		

20. Fill in your **GPS** data for all other sites.

21. Highlight the **G2** and **H2** cells together and copy the contents, press the "***Ctrl + C***".

22. Scroll down to cell **G3** and paste the formula, "***Ctrl + V***".

a) Fill all the way down to **G10**.

Your database should look like below.

G10 =LOOKUP(E10,Metadata!$A$2:$A$57,Metadata!$C$2:$C$57)

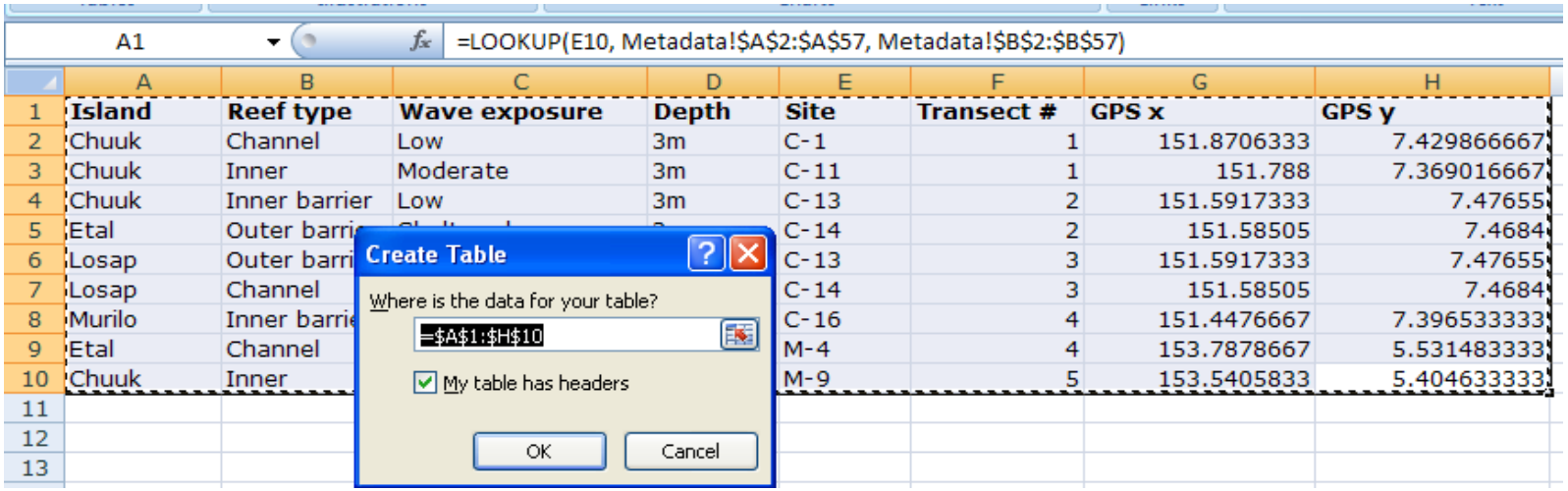
	A	B	C	D	E	F	G	H
1	**Island**	**Reef type**	**Wave exposure**	**Depth**	**Site**	**Transect #**	**GPS x**	**GPS y**
2	Chuuk	Channel	Low	3m	C-1	1	151.8706333	7.429866667
3	Chuuk	Inner	Moderate	3m	C-11	1	151.788	7.369016667
4	Chuuk	Inner barrier	Low	3m	C-13	2	151.5917333	7.47655
5	Etal	Outer barrier	Sheltered	3m	C-14	2	151.58505	7.4684
6	Losap	Outer barrier	Moderate	3m	C-13	3	151.5917333	7.47655
7	Losap	Channel	High	10m	C-14	3	151.58505	7.4684
8	Murilo	Inner barrier	Low	10m	C-16	4	151.4476667	7.396533333
9	Etal	Channel	Moderate	10m	M-4	4	153.7878667	5.531483333
10	Chuuk	Inner	Moderate	10m	M-9	5	153.5405833	5.404633333
11								

Now that we have everything in order, we are ready to formalize our database into an Excel "**list**" function.

23. Highlight all cells where data exists, **A1** to **H10**.

a) Click on the "**Insert**" main tab for Excel, on the sub-menu **click** on "**table**".

You should have a dialog box appear as below.



b) Make sure the box for "**My table has headers**" is checked, and click **OK**.

24. Click in cell **A11**.

a) Select any island of you like from the drop down menu.

	A	B	C	D	E	F	G	H
1	Island	Reef type	Wave exposure	Depth	Site	Transect #	GPS x	GPS y
2	Chuuk	Channel	Low	3m	C-1	1	151.8706333	7.429866667
3	Chuuk	Inner	Moderate	3m	C-11	1	151.788	7.369016667
4	Chuuk	Inner barrier	Low	3m	C-13	2	151.5917333	7.47655
5	Etal	Outer barrier	Sheltered	3m	C-14	2	151.58505	7.4684
6	Losap	Outer barrier	Moderate	3m	C-13	3	151.5917333	7.47655
7	Losap	Channel	High	10m	C-14	3	151.58505	7.4684
8	Murilo	Inner barrier	Low	10m	C-16	4	151.4476667	7.396533333
9	Etal	Channel	Moderate	10m	M-4	4	153.7878667	5.531483333
10	Chuuk	Inner	Moderate	10m	M-9	5	153.5405833	5.404633333
11								

25. Do the same with "**reef type**", "**wave exposure**", "**depth**", "**site**", and "**transect name**".

Note: GPS data is automatically entered for you. This is because of our lookup table.

Time to enter our ecological survey data of the macroinvertebrate abundances. There are two approaches commonly used. The first is especially relevant for count data that has been collected without individual sizes, such as counting the numbers of sea cucumbers but not measuring the length of each one.

26. Highlight cell **I1**.

a) Type in the name of one common sea cucumber, ***Holothuria atra***, then push **enter**.

Note: excel automatically extends your "list" to include column I.

27. Enter numbers of sea cucumbers encountered for each transect surveyed. You can just enter values of your choosing.

	A	B	C	D	E	F	G	H	I
1	Island	Reef type	Wave exposure	Depth	Site	Transect #	GPS x	GPS y	Holothu
2	Chuuk	Channel	Low	3m	C-1	1	151.8706333	7.429866667	25
3	Chuuk	Inner	Moderate	3m	C-11	1	151.788	7.369016667	33
4	Chuuk	Inner barrier	Low	3m	C-13	2	151.5917333	7.47655	2
5	Etal	Outer barrier	Sheltered	3m	C-14	2	151.58505	7.4684	3
6	Losap	Outer barrier	Moderate	3m	C-13	3	151.5917333	7.47655	4
7	Losap	Channel	High	10m	C-14	3	151.58505	7.4684	5
8	Murilo	Inner barrier	Low	10m	C-16	4	151.4476667	7.396533333	6
9	Etal	Channel	Moderate	10m	M-4	4	153.7878667	5.531483333	66
10	Chuuk	Inner	Moderate	10m	M-9	5	153.5405833	5.404633333	54
11	Satawan	Outer barrier	High	3m	C-12	3	151.5751	7.471166667	33

It is very straightforward how to continue to enter data in this manner, one can keep on adding species in the columns to the right of the Excel "list".

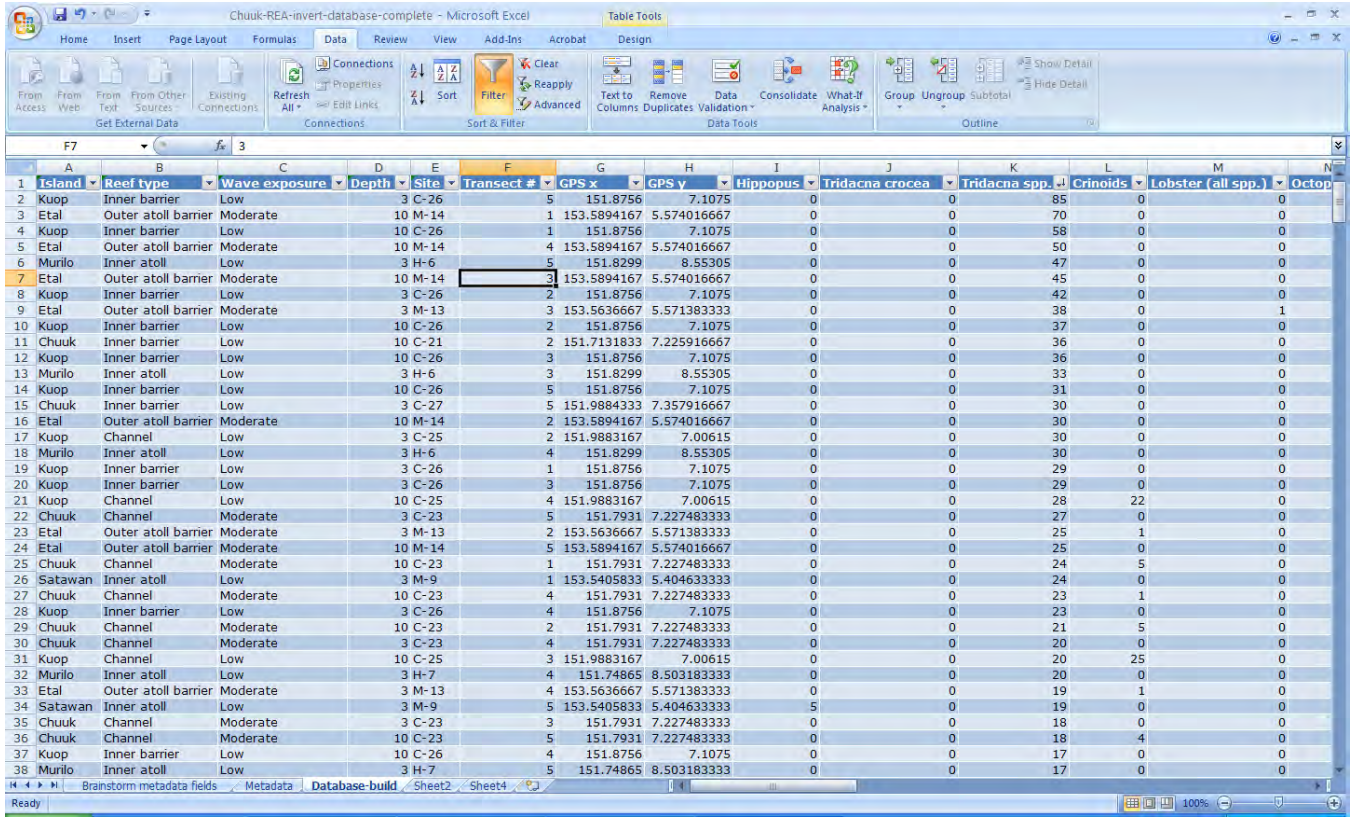
Finish exercise 1, save your Excel file for future reference.

Exercise 2 – Manipulating, Managing, Working with, and Visualizing a Database

1. **Open** the file "**Chuuk-REA-invert-database-complete**".
   a. Click on the sheet "Database-build".

   Here, you will find the same database we just built, however, now it is populated with 520 transects of macroinvertebrate data abundance estimates that were collected during the Chuuk REA. Examine the database, especially look at the organization. The data are sorted by Island, Site, and Depth. You can re-sort the data by using the column headers, and clicking on the dropdown arrow next to the column heading.

2. **Click** on cell **K2** "***Tridacna* spp**.".
   a. **Sort** from "**largest to smallest**".



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Island	Reef type	Wave exposure	Depth	Site	Transect #	GPS x	GPS y	Hippopus	Tridacna crocea	Tridacna spp.	Crinoids	Lobster (all spp.)	Octop
2	Kuop	Inner barrier	Low	3	C-26	5	151.8756	7.1075	0	0	85	0	0	
3	Etal	Outer atoll barrier	Moderate	10	M-14	1	153.5894167	5.574016667	0	0	70	0	0	
4	Kuop	Inner barrier	Low	10	C-26	1	151.8756	7.1075	0	0	58	0	0	
5	Etal	Outer atoll barrier	Moderate	10	M-14	4	153.5894167	5.574016667	0	0	50	0	0	
6	Murilo	Inner atoll	Low	3	H-6	5	151.8299	8.55305	0	0	47	0	0	
7	Etal	Outer atoll barrier	Moderate	10	M-14	3	153.5894167	5.574016667	0	0	45	0	0	
8	Kuop	Inner barrier	Low	3	C-26	2	151.8756	7.1075	0	0	42	0	0	
9	Etal	Outer atoll barrier	Moderate	3	M-13	3	153.5636667	5.571383333	0	0	38	0	1	
10	Kuop	Inner barrier	Low	10	C-26	2	151.8756	7.1075	0	0	37	0	0	
11	Chuuk	Inner barrier	Low	10	C-21	2	151.7131833	7.225916667	0	0	36	0	0	
12	Kuop	Inner barrier	Low	10	C-26	3	151.8756	7.1075	0	0	36	0	0	
13	Murilo	Inner atoll	Low	3	H-6	3	151.8299	8.55305	0	0	33	0	0	
14	Kuop	Inner barrier	Low	10	C-26	5	151.8756	7.1075	0	0	31	0	0	
15	Chuuk	Inner barrier	Low	3	C-27	5	151.9884333	7.357916667	0	0	30	0	0	
16	Etal	Outer atoll barrier	Moderate	10	M-14	2	153.5894167	5.574016667	0	0	30	0	0	
17	Kuop	Channel	Low	3	C-25	2	151.9883167	7.00615	0	0	30	0	0	
18	Murilo	Inner atoll	Low	3	H-6	4	151.8299	8.55305	0	0	30	0	0	
19	Kuop	Inner barrier	Low	3	C-26	1	151.8756	7.1075	0	0	29	0	0	
20	Kuop	Inner barrier	Low	3	C-26	3	151.8756	7.1075	0	0	29	0	0	
21	Kuop	Channel	Low	10	C-25	4	151.9883167	7.00615	0	0	28	22	0	
22	Chuuk	Channel	Moderate	3	C-23	5	151.7931	7.227483333	0	0	27	0	0	
23	Etal	Outer atoll barrier	Moderate	3	M-13	2	153.5636667	5.571383333	0	0	25	1	0	
24	Etal	Outer atoll barrier	Moderate	10	M-14	5	153.5894167	5.574016667	0	0	25	0	0	
25	Chuuk	Channel	Moderate	10	C-23	1	151.7931	7.227483333	0	0	24	5	0	
26	Satawan	Inner atoll	Low	3	M-9	1	153.5405833	5.404633333	0	0	24	0	0	
27	Chuuk	Channel	Moderate	10	C-23	4	151.7931	7.227483333	0	0	23	1	0	
28	Kuop	Inner barrier	Low	3	C-26	4	151.8756	7.1075	0	0	23	0	0	
29	Chuuk	Channel	Moderate	10	C-23	2	151.7931	7.227483333	0	0	21	5	0	
30	Chuuk	Channel	Moderate	3	C-23	4	151.7931	7.227483333	0	0	20	0	0	
31	Kuop	Channel	Low	10	C-25	3	151.9883167	7.00615	0	0	20	25	0	
32	Murilo	Inner atoll	Low	3	H-7	4	151.74865	8.503183333	0	0	20	0	0	
33	Etal	Outer atoll barrier	Moderate	3	M-13	4	153.5636667	5.571383333	0	0	19	1	0	
34	Satawan	Inner atoll	Low	3	M-9	5	153.5405833	5.404633333	5	0	19	0	0	
35	Chuuk	Channel	Moderate	3	C-23	3	151.7931	7.227483333	0	0	18	0	0	
36	Chuuk	Channel	Moderate	10	C-23	5	151.7931	7.227483333	0	0	18	4	0	
37	Kuop	Inner barrier	Low	10	C-26	4	151.8756	7.1075	0	0	17	0	0	
38	Murilo	Inner atoll	Low	3	H-7	5	151.74865	8.503183333	0	0	17	0	0	

You can get a general understanding this way, for instance, that the atolls hold more large clams (grouped as Tridacna spp.), as compared with Chuuk. And, in particular, Kuop seems to appear many times at the top of the list.

3. Now, do the same sorting for the common sea cucumber ***Holothuria atra***.

Which island consistently holds the greatest abundance of this common sea cucumber?

Lets return the database back to its original form.

4. Click on the "**Depth**"
   a. Sort "**smallest to largest**".
5. Click on **site**
   b. Sort "**A to Z**".
6. Click on "**Island**"
   c. Sort "**A to Z**".

You can notice this is exactly how the database looked when we first opened it.
Now, we will add some additive, summary columns that will help us to better visualize our results. Notice that columns I, J, and K all refer to "clams". Lets add a column to help summarize the abundance of all clams together.

7. Click on column **L** "**Crinoids**", right after the last column with clam names.
8. Right click the mouse and **select** "***insert***".

*Notice a new column appears called "**Column 1**".*

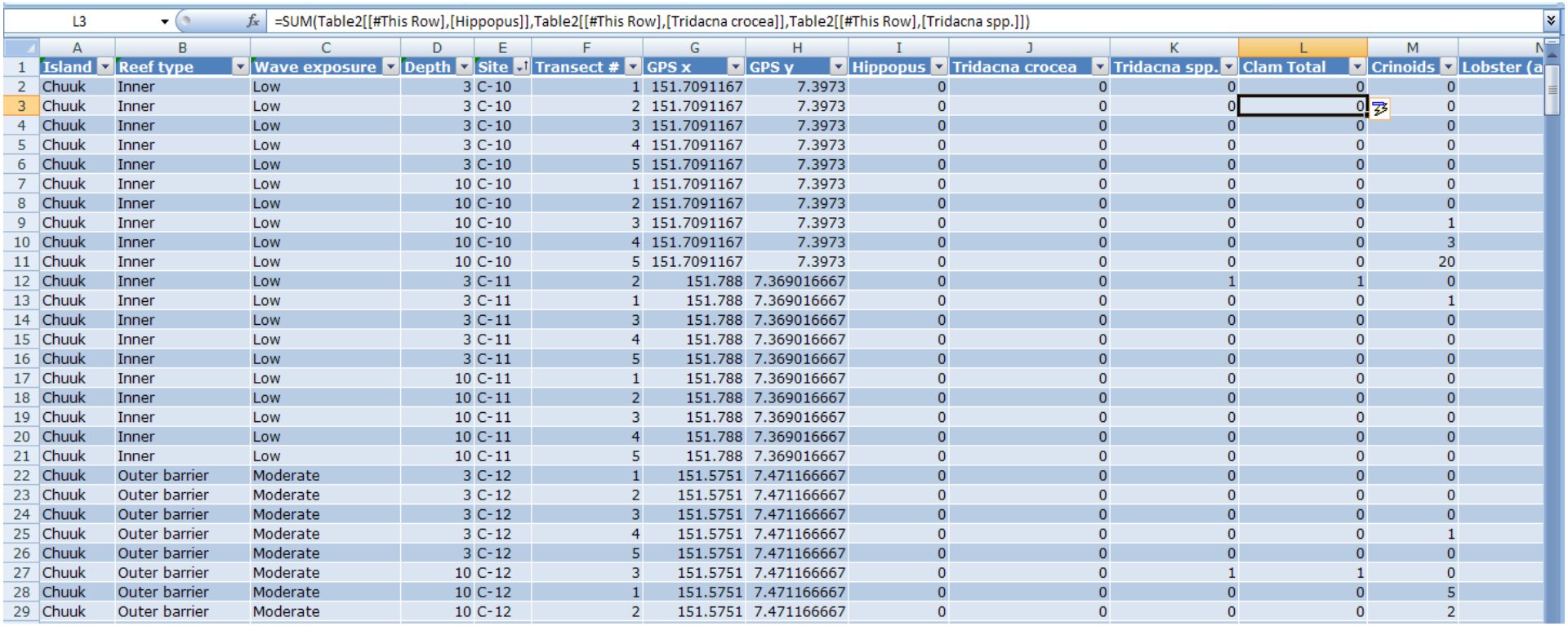
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Island	Reef type	Wave exposure	Depth	Site	Transect #	GPS x	GPS y	Hippopus	Tridacna crocea	Tridacna spp.	Column1	noids	Lobster (a
2	Chuuk	Inner	Low	3	C-10	1	151.7091167	7.3973	0	0	0		0	
3	Chuuk	Inner	Low	3	C-10	2	151.7091167	7.3973	0	0	0		0	
4	Chuuk	Inner	Low	3	C-10	3	151.7091167	7.3973	0	0	0		0	
5	Chuuk	Inner	Low	3	C-10	4	151.7091167	7.3973	0	0	0		0	
6	Chuuk	Inner	Low	3	C-10	5	151.7091167	7.3973	0	0	0		0	
7	Chuuk	Inner	Low	10	C-10	1	151.7091167	7.3973	0	0	0		0	
8	Chuuk	Inner	Low	10	C-10	2	151.7091167	7.3973	0	0	0		0	
9	Chuuk	Inner	Low	10	C-10	3	151.7091167	7.3973	0	0	0		1	
10	Chuuk	Inner	Low	10	C-10	4	151.7091167	7.3973	0	0	0		3	
11	Chuuk	Inner	Low	10	C-10	5	151.7091167	7.3973	0	0	0		20	
12	Chuuk	Inner	Low	3	C-11	2	151.788	7.369016667	0	0	1		0	
13	Chuuk	Inner	Low	3	C-11	1	151.788	7.369016667	0	0	0		1	
14	Chuuk	Inner	Low	3	C-11	3	151.788	7.369016667	0	0	0		0	

   a. Change the name to "***Clam Total***".

9. Now, **highlight** cell ***L2***. **Write** "=sum(" *(to conduct an automated sum function in excel.)*

- **b. Click** on the cell ***I2***, place a comma(,).
- **c. Click** on ***J2***, add another comma,
- **d. Click** on ***K2,*** finish with a closed parenthesis ")".
- **e. Press *enter***.

Excel fills in a sum function for the entire database automatically. This column is now the total abundance of all three clam categories, and can be used as a summary.



L3 =SUM(Table2[[#This Row],[Hippopus]],Table2[[#This Row],[Tridacna crocea]],Table2[[#This Row],[Tridacna spp.]])

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Island	Reef type	Wave exposure	Depth	Site	Transect #	GPS x	GPS y	Hippopus	Tridacna crocea	Tridacna spp.	Clam Total	Crinoids	Lobster (a
2	Chuuk	Inner	Low	3	C-10	1	151.7091167	7.3973	0	0	0	0	0	
3	Chuuk	Inner	Low	3	C-10	2	151.7091167	7.3973	0	0	0	0	0	
4	Chuuk	Inner	Low	3	C-10	3	151.7091167	7.3973	0	0	0	0	0	
5	Chuuk	Inner	Low	3	C-10	4	151.7091167	7.3973	0	0	0	0	0	
6	Chuuk	Inner	Low	3	C-10	5	151.7091167	7.3973	0	0	0	0	0	
7	Chuuk	Inner	Low	10	C-10	1	151.7091167	7.3973	0	0	0	0	0	
8	Chuuk	Inner	Low	10	C-10	2	151.7091167	7.3973	0	0	0	0	0	
9	Chuuk	Inner	Low	10	C-10	3	151.7091167	7.3973	0	0	0	0	1	
10	Chuuk	Inner	Low	10	C-10	4	151.7091167	7.3973	0	0	0	0	3	
11	Chuuk	Inner	Low	10	C-10	5	151.7091167	7.3973	0	0	0	0	20	
12	Chuuk	Inner	Low	3	C-11	2	151.788	7.369016667	0	0	1	1	0	
13	Chuuk	Inner	Low	3	C-11	1	151.788	7.369016667	0	0	0	0	1	
14	Chuuk	Inner	Low	3	C-11	3	151.788	7.369016667	0	0	0	0	0	
15	Chuuk	Inner	Low	3	C-11	4	151.788	7.369016667	0	0	0	0	0	
16	Chuuk	Inner	Low	3	C-11	5	151.788	7.369016667	0	0	0	0	0	
17	Chuuk	Inner	Low	10	C-11	1	151.788	7.369016667	0	0	0	0	0	
18	Chuuk	Inner	Low	10	C-11	2	151.788	7.369016667	0	0	0	0	0	
19	Chuuk	Inner	Low	10	C-11	3	151.788	7.369016667	0	0	0	0	0	
20	Chuuk	Inner	Low	10	C-11	4	151.788	7.369016667	0	0	0	0	0	
21	Chuuk	Inner	Low	10	C-11	5	151.788	7.369016667	0	0	0	0	0	
22	Chuuk	Outer barrier	Moderate	3	C-12	1	151.5751	7.471166667	0	0	0	0	0	
23	Chuuk	Outer barrier	Moderate	3	C-12	2	151.5751	7.471166667	0	0	0	0	0	
24	Chuuk	Outer barrier	Moderate	3	C-12	3	151.5751	7.471166667	0	0	0	0	0	
25	Chuuk	Outer barrier	Moderate	3	C-12	4	151.5751	7.471166667	0	0	0	0	1	
26	Chuuk	Outer barrier	Moderate	3	C-12	5	151.5751	7.471166667	0	0	0	0	0	
27	Chuuk	Outer barrier	Moderate	10	C-12	3	151.5751	7.471166667	0	0	1	1	0	
28	Chuuk	Outer barrier	Moderate	10	C-12	1	151.5751	7.471166667	0	0	0	0	5	
29	Chuuk	Outer barrier	Moderate	10	C-12	2	151.5751	7.471166667	0	0	0	0	2	

Next, we will do the same for sea cucumbers. Column AB has the name of the last sea cucumber, "Thelonota anax".

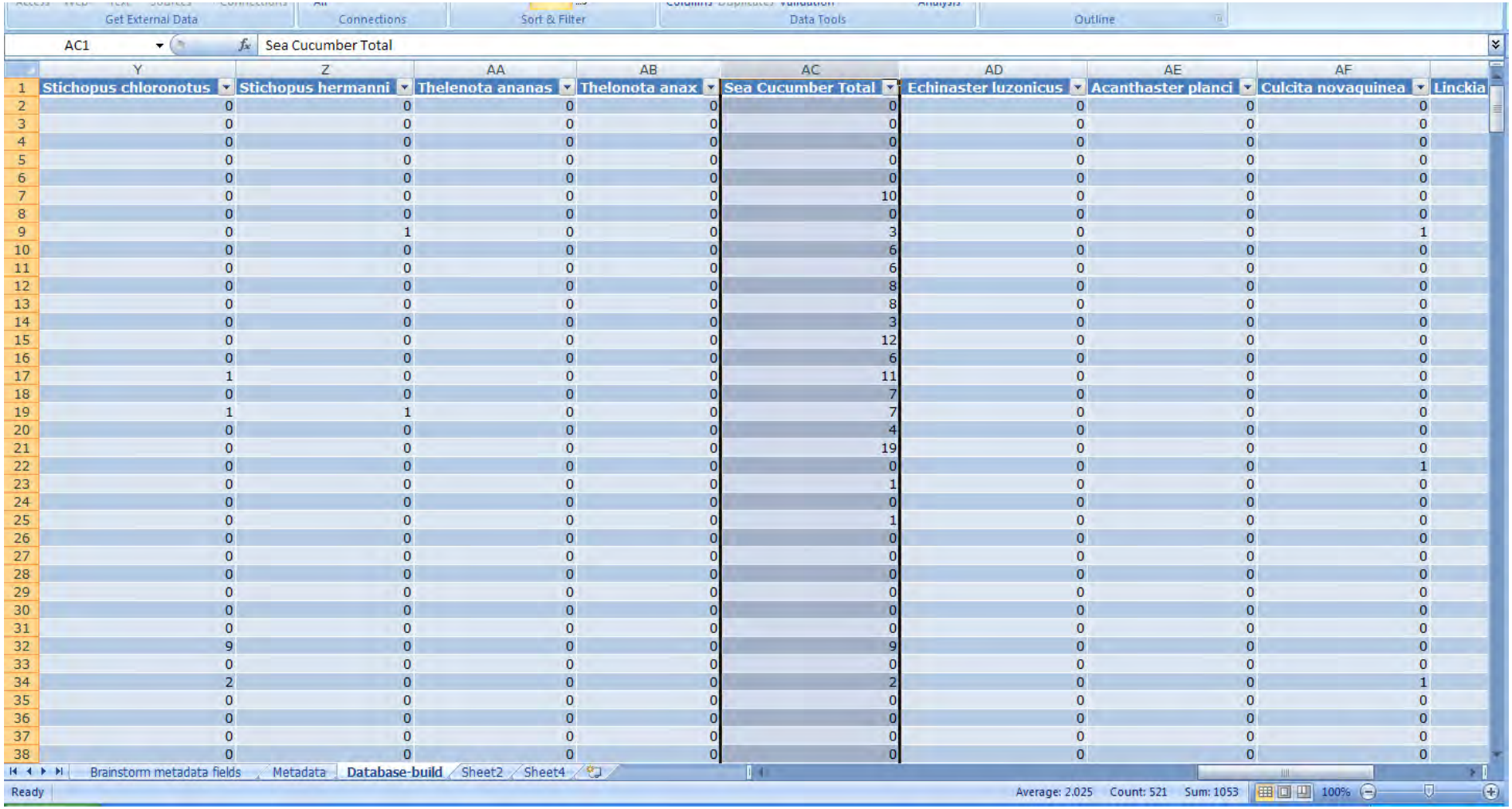
10. Click on the next column, ***AC***, and **right click**, and again "***insert***".

- **a. Name** this ***Sea Cucumber Total***.

11. Do the **sum function** for excel, ensure that all sea cucumbers are included, columns P through AC.

*(**Note**: Instead of clicking individual cells, you can drag the excel cursor across all cells if you like.)*

Your spreadsheet should look like below.

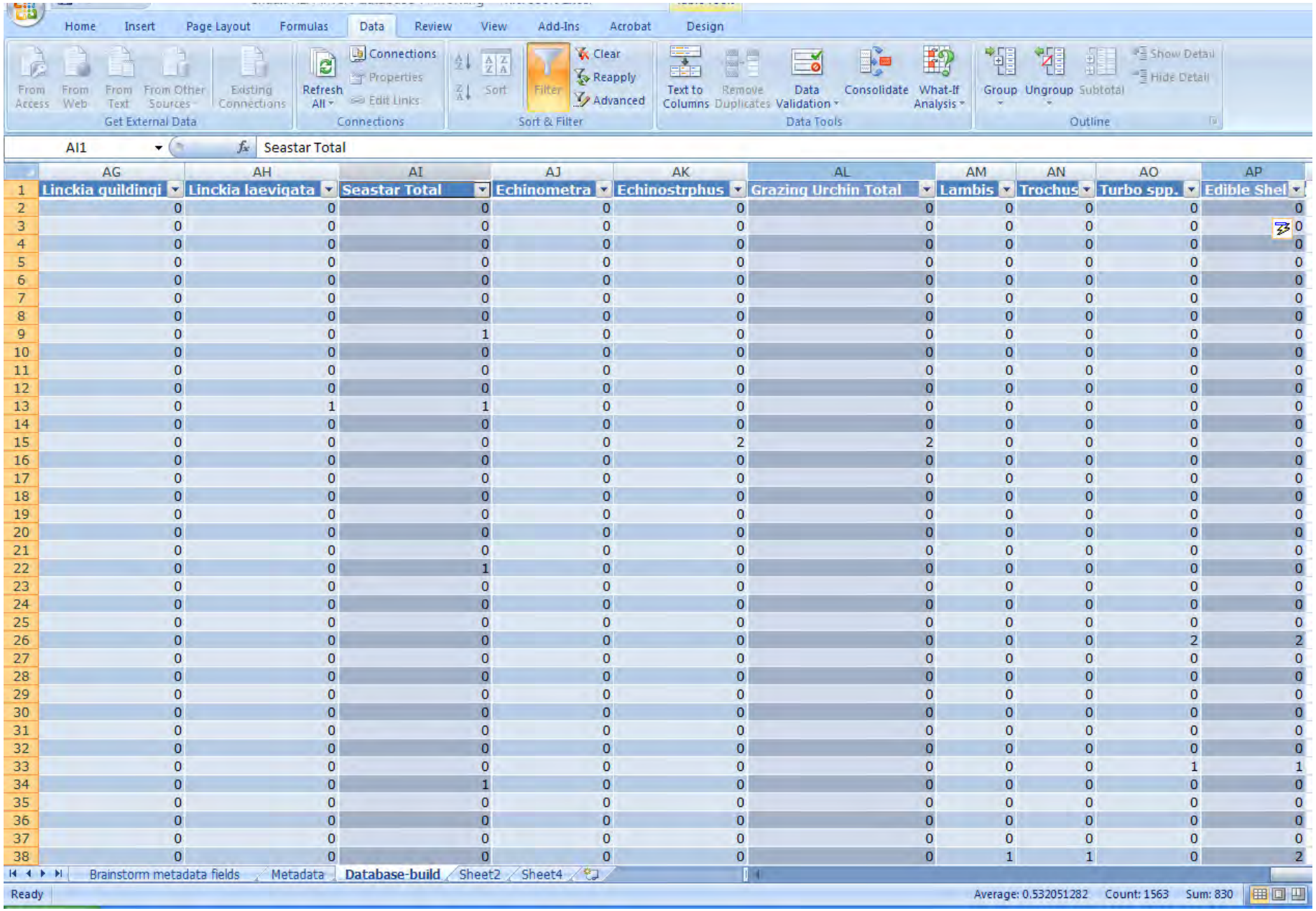


	Y	Z	AA	AB	AC	AD	AE	AF	
1	Stichopus chloronotus	Stichopus hermanni	Thelenota ananas	Thelonota anax	Sea Cucumber Total	Echinaster luzonicus	Acanthaster planci	Culcita novaguinea	Linckia
2	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	
6	0	0	0	0	0	0	0	0	
7	0	0	0	0	10	0	0	0	
8	0	0	0	0	0	0	0	0	
9	0	1	0	0	3	0	0	1	
10	0	0	0	0	6	0	0	0	
11	0	0	0	0	6	0	0	0	
12	0	0	0	0	8	0	0	0	
13	0	0	0	0	8	0	0	0	
14	0	0	0	0	3	0	0	0	
15	0	0	0	0	12	0	0	0	
16	0	0	0	0	6	0	0	0	
17	1	0	0	0	11	0	0	0	
18	0	0	0	0	7	0	0	0	
19	1	1	0	0	7	0	0	0	
20	0	0	0	0	4	0	0	0	
21	0	0	0	0	19	0	0	0	
22	0	0	0	0	0	0	0	1	
23	0	0	0	0	1	0	0	0	
24	0	0	0	0	0	0	0	0	
25	0	0	0	0	1	0	0	0	
26	0	0	0	0	0	0	0	0	
27	0	0	0	0	0	0	0	0	
28	0	0	0	0	0	0	0	0	
29	0	0	0	0	0	0	0	0	
30	0	0	0	0	0	0	0	0	
31	0	0	0	0	0	0	0	0	
32	9	0	0	0	9	0	0	0	
33	0	0	0	0	0	0	0	0	
34	2	0	0	0	2	0	0	1	
35	0	0	0	0	0	0	0	0	
36	0	0	0	0	0	0	0	0	
37	0	0	0	0	0	0	0	0	
38	0	0	0	0	0	0	0	0	

12. **Repeat** process for:
    a. ***Seastars*** – Columns AD through AH contain names of seastars
    b. ***Grazing urchins*** - Columns AJ and AK contain grazing urchins.
13. **Repeat** process for ***edible shells*** too. ***(Note:** Here, you can just click in the cell AP1 and type "Edible Shell Total")*

a. Do the same ***sum function***.

b. See below for confirmation.



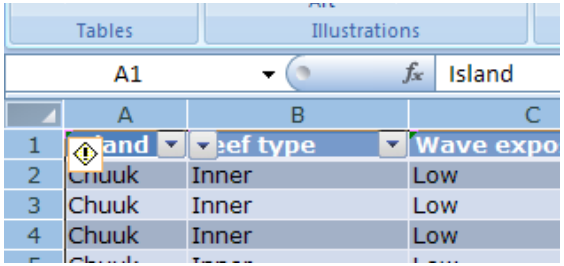
	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP
1	Linckia guildingi	Linckia laevigata	Seastar Total	Echinometra	Echinostrphus	Grazing Urchin Total	Lambis	Trochus	Turbo spp.	Edible Shel
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	1	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	1	1	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	2	2	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
22	0	0	1	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	2	2
27	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	1	1
34	0	0	1	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	1	1	0	2

That ends our basic database manipulation, you can review the steps and logically think of other ways to do similar things.

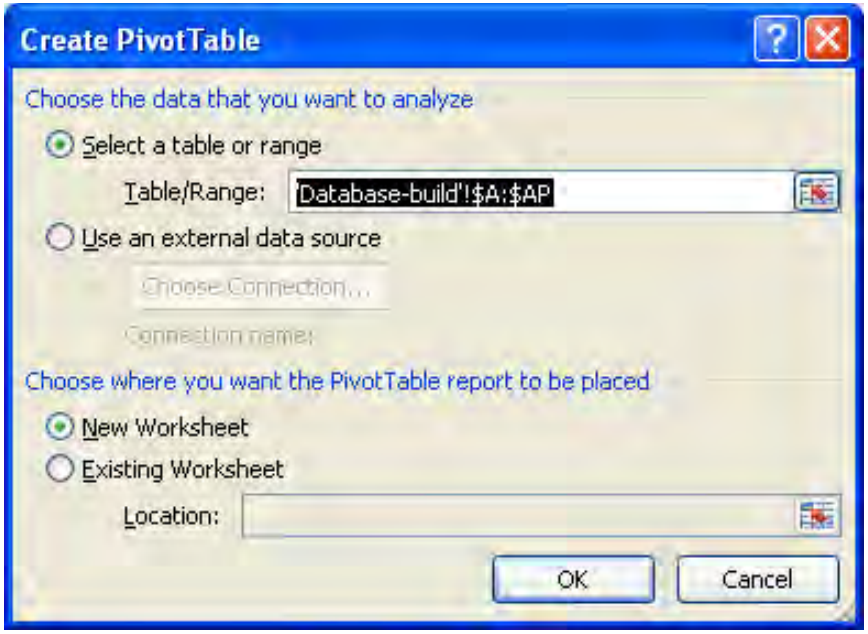
Now, we will begin to visualize the dataset using **Excel's Pivot Table**.
In order to set up a Pivot Table, you first need to highlight the cells that define the table.

14. To the upper left of cell ***A1***, there is a small box with a ***diagonal arrow***.

- **a. Click** on that ***box*** *(all cells in the database are automatically highlighted)*

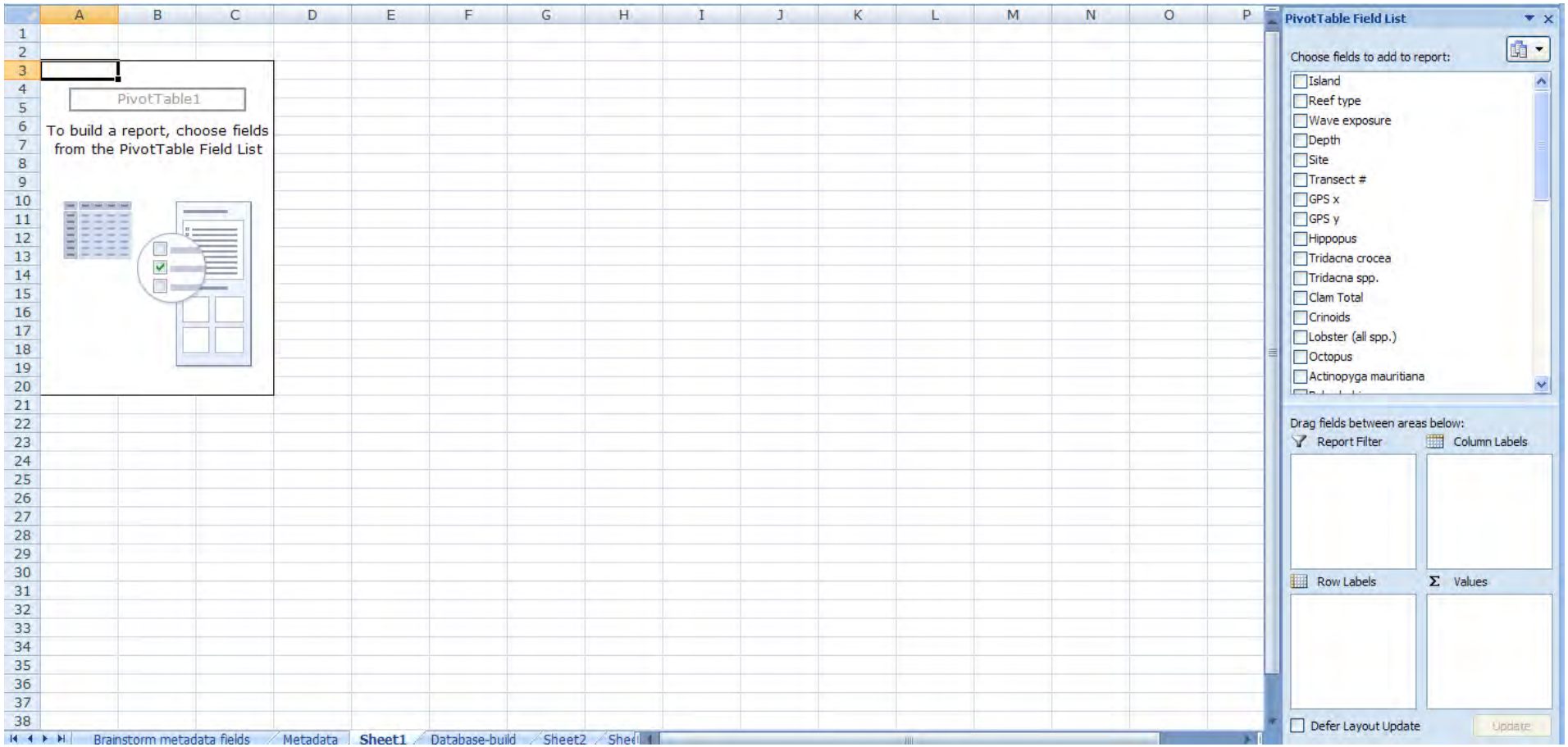


- **b. Click** on the "***Insert Tab***" of Excels main menu, and
- **c. Click** on ***Pivot Table***.
- **d.** The ***table/range*** should match, and ensure that "***New Worksheet***" is selected.



- **e. Click *OK***.

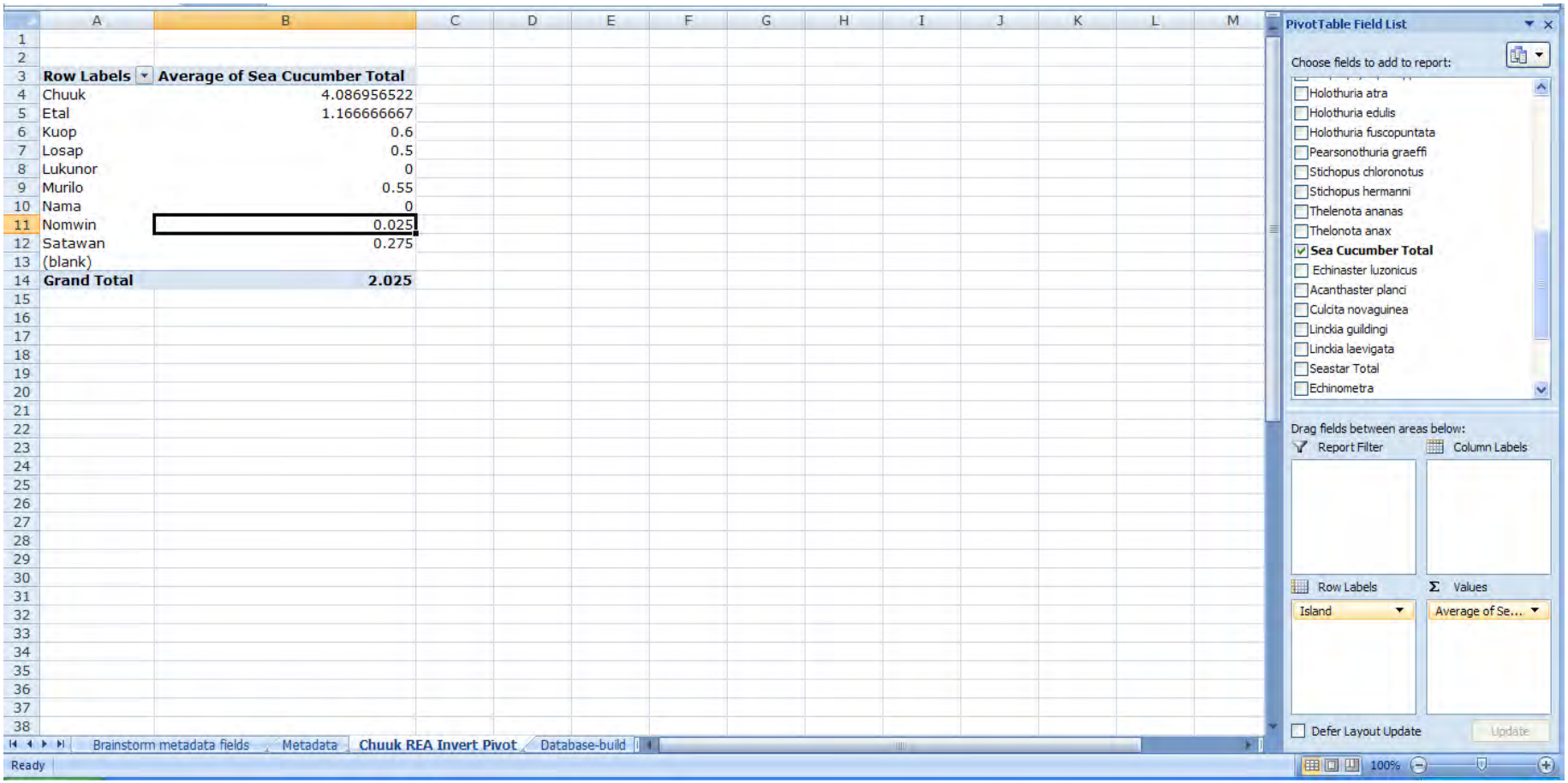
A new sheet (see below) should be created between "**Metadata**" and "**Database-build**" that is called "**Sheet 1**". You can right click and rename it to "**Chuuk REA Invert Pivot**". **Click** back inside the "**Pivot table area"** in the upper left. With **Pivot Table** you can make summary tables and graphs easily and quickly. The first thing we will do is take a simple look at sea cucumber abundances by island.



15. **Click** and **Drag** the "***Island***" Box from the "***Pivot Table Field list"*** on the right and place it down in the "***Row Labels***".
16. **Click** and **Drag** "***Sea Cucumber Total***" box from the "***Pivot table field list"*** (*hint, you need to scroll down to find it*), and **place** it under "***values"***
17. **Click** one time on the "***Count of Sea Cucumber Total***" box under values, a sub-menu should pop-up.
    a. **Click** on the "***Value Field Setting***" - *Notice count is selected, but we want to examine average values found on each transect.*

b. Click on "***Average***".

c. *See below for a confirmation of these steps*



Row Labels	Average of Sea Cucumber Total
Chuuk	4.086956522
Etal	1.166666667
Kuop	0.6
Losap	0.5
Lukunor	0
Murilo	0.55
Nama	0
Nomwin	0.025
Satawan	0.275
(blank)	
Grand Total	**2.025**

Lets also view the standard deviations to understand how the data was spread among the surveyed transects.

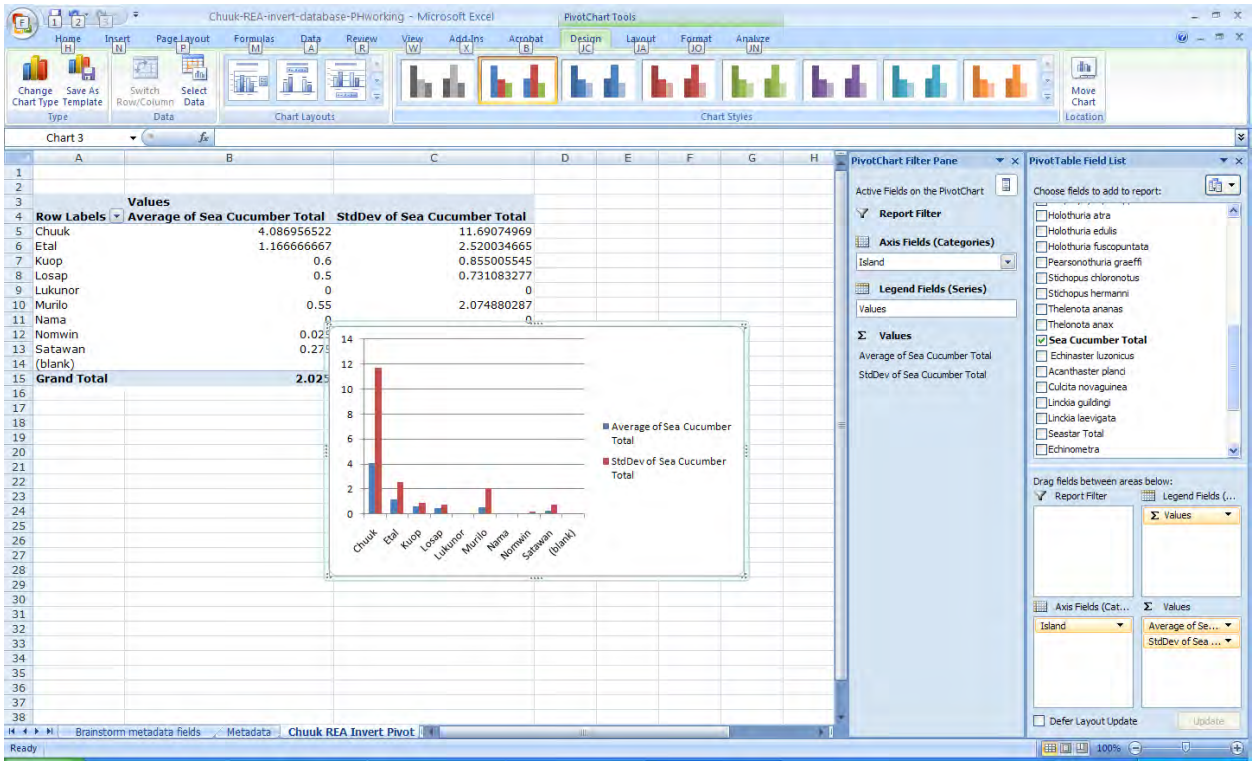
18. Click and **drag** the "***Sea Cucumber Total***" box from on top below the existing "***Average of Sea Cucumber***" in ***Values***.

a. Click the new "***Count of Sea Cucumber Total***" box and again choose "***Value Field Setting***".

b. Scroll down on the pop-up menu and choose "***StdDev***".

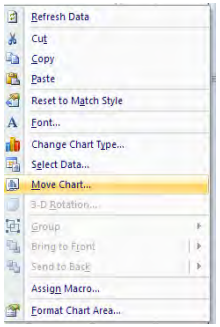
Now you have averages and standard deviations side by side, lets view this graphically.

19. **Click** on any cell in the ***Pivot Table*** *(the new data table on the upper left of the sheet)*
    a. **Click** on the "***Insert***" main menu tab in Excel. (*You can see a lot of options here, we want to look at simple "Column" charts)*
    b. **Click** on "***Column***", and **select** the "***first graph option"*** in the top left.

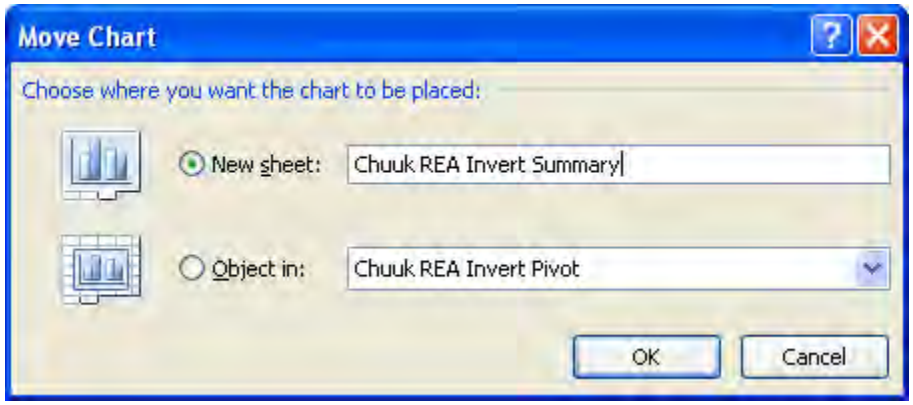


Let's move the chart to a new sheet for simplicity.

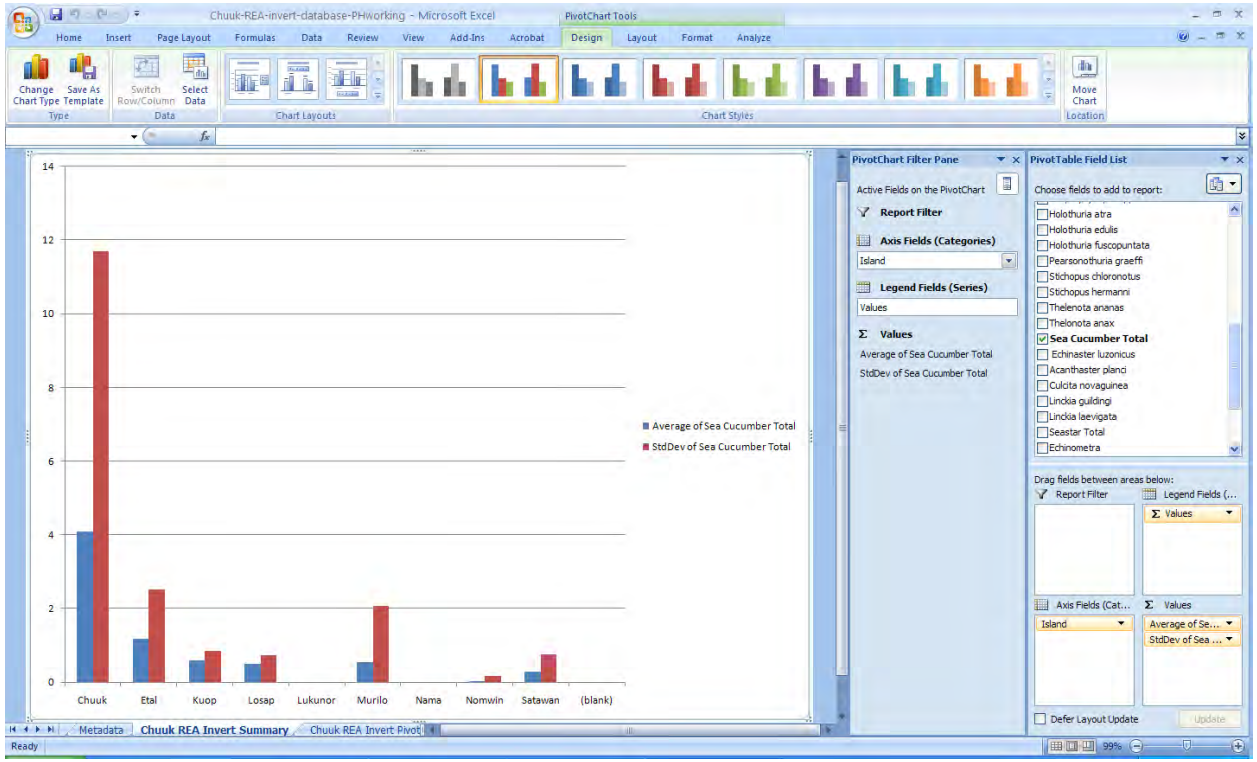
20. **Right click** in the chart and select "***Move Chart***".



21. Select "***New Sheet***" and **rename** the chart "***Chuuk REA Invert Summary***".



A new sheet is created and our desired summary is easily seen and understood.

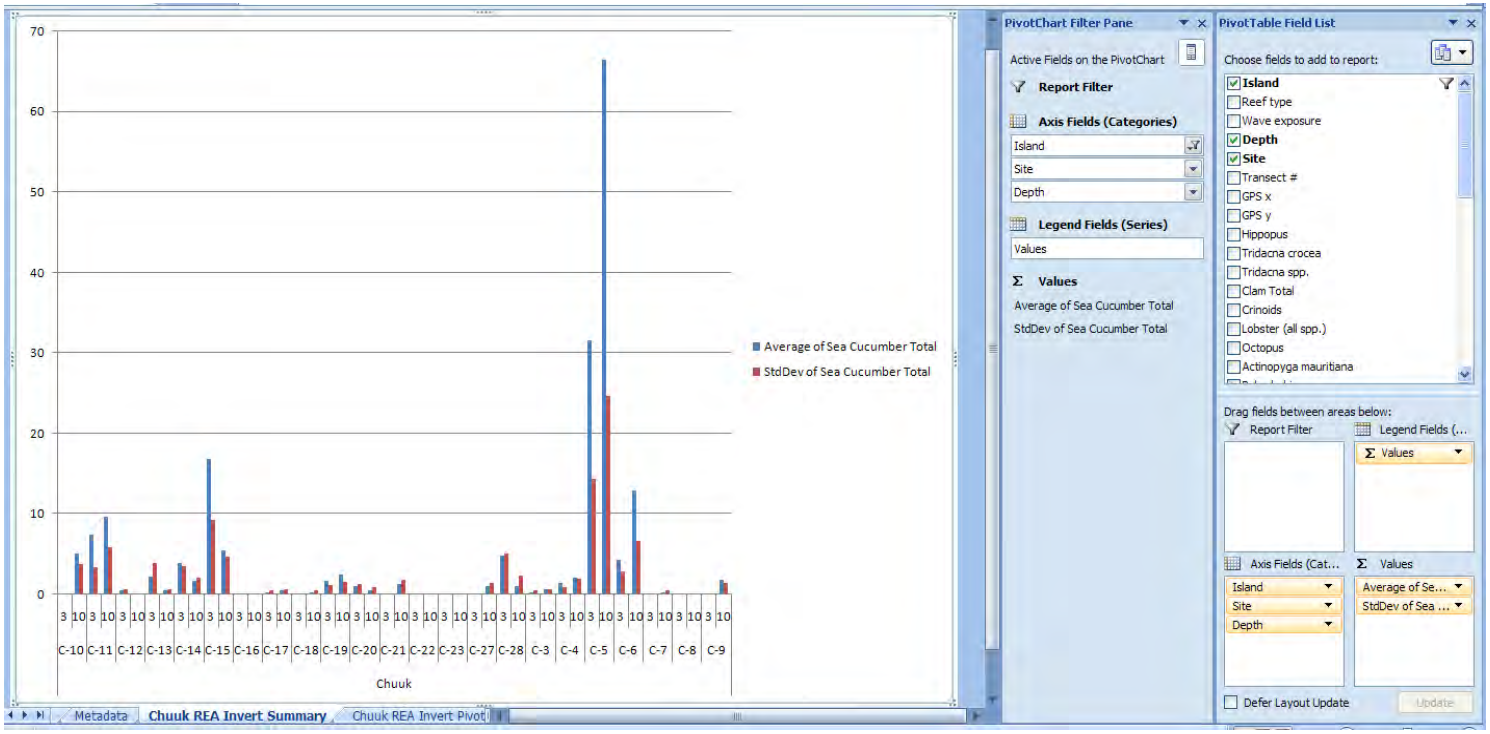


Now, we can take a moment to reflect upon what the data is telling us. First, on average, there was no site surveyed in Chuuk that had more than 4 sea cucumbers per 5-minute swim, a very low value compared with other REA reports conducted in similar habitats and depths. Second, Chuuk has the greatest abundance of sea cucumbers, a consequence of the high islands located in Chuuk Lagoon, providing suspended particulate matter to the lagoon through the deposition of terrestrial organic matter. These trends are expected. Third, the outer islands all have very low abundances; in fact at some none were recorded. Fourth, in all instances the standard deviation is greater than the average. This informs us right away that our statistical power to detect change over time in sea cucumber abundances is low for the entire island level. However, our goals are to understand change at the individual site level. So we will see how the data improve our understanding of the distribution of sea cucumbers around Chuuk only.

Notice you can manipulate the Pivot Table in chart mode with Excel as well as table mode. These next steps could be done on the "Chuuk REA Invert Summary" graph sheet, or the "Chuuk REA Invert Pivot" table sheet. Keep on the graph sheet for now.

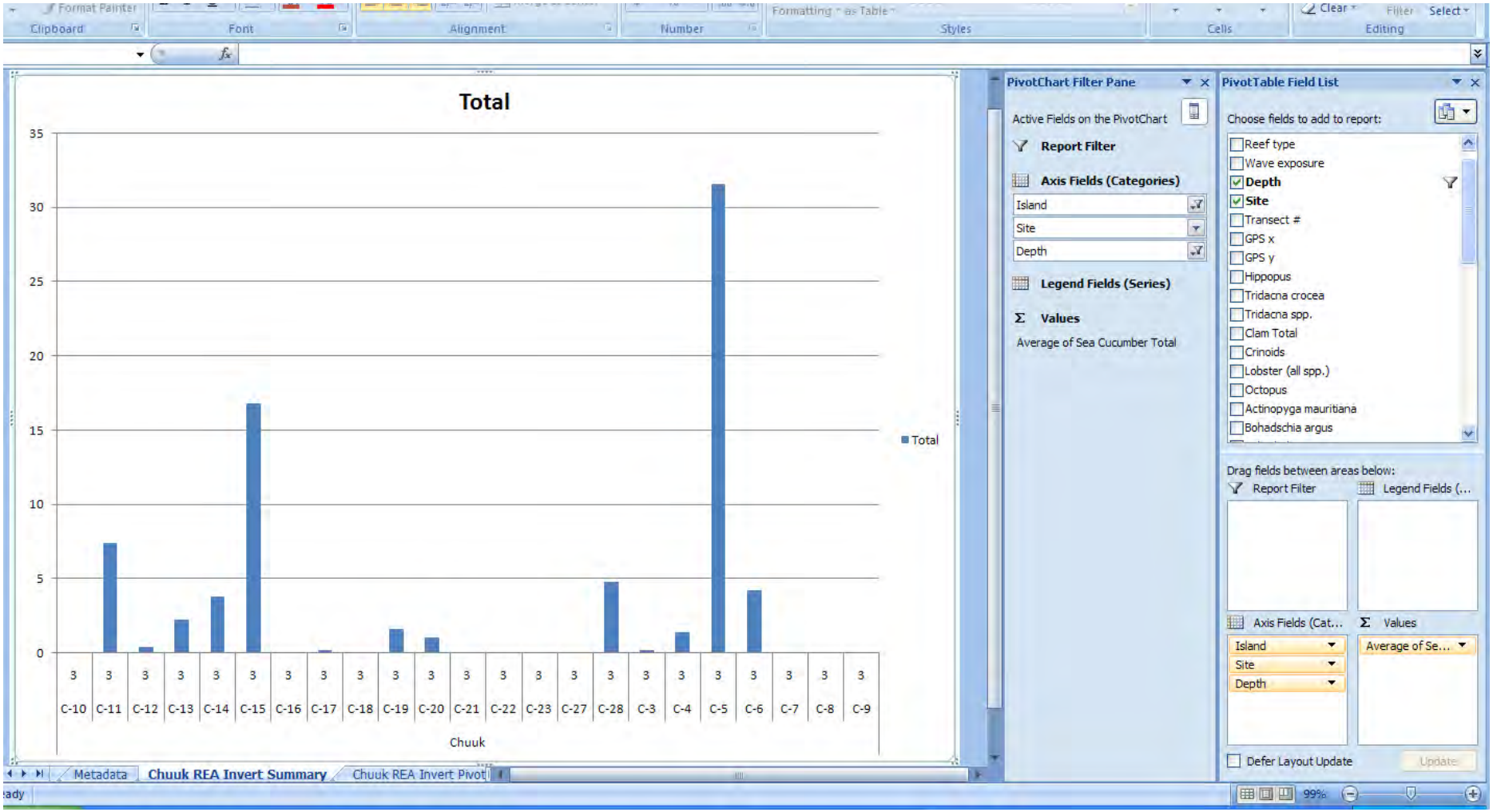
22. Under the "***PivotChart Filter Pane***" window **click** on the ***drop down menu*** for "***Island***".

- **a. Uncheck** all islands except for Chuuk.
- **b.** On the "***PivotTable field list***" **drag** "***Site***" and "***Depth***" below "***Island***".
- **c.** Confirm below.

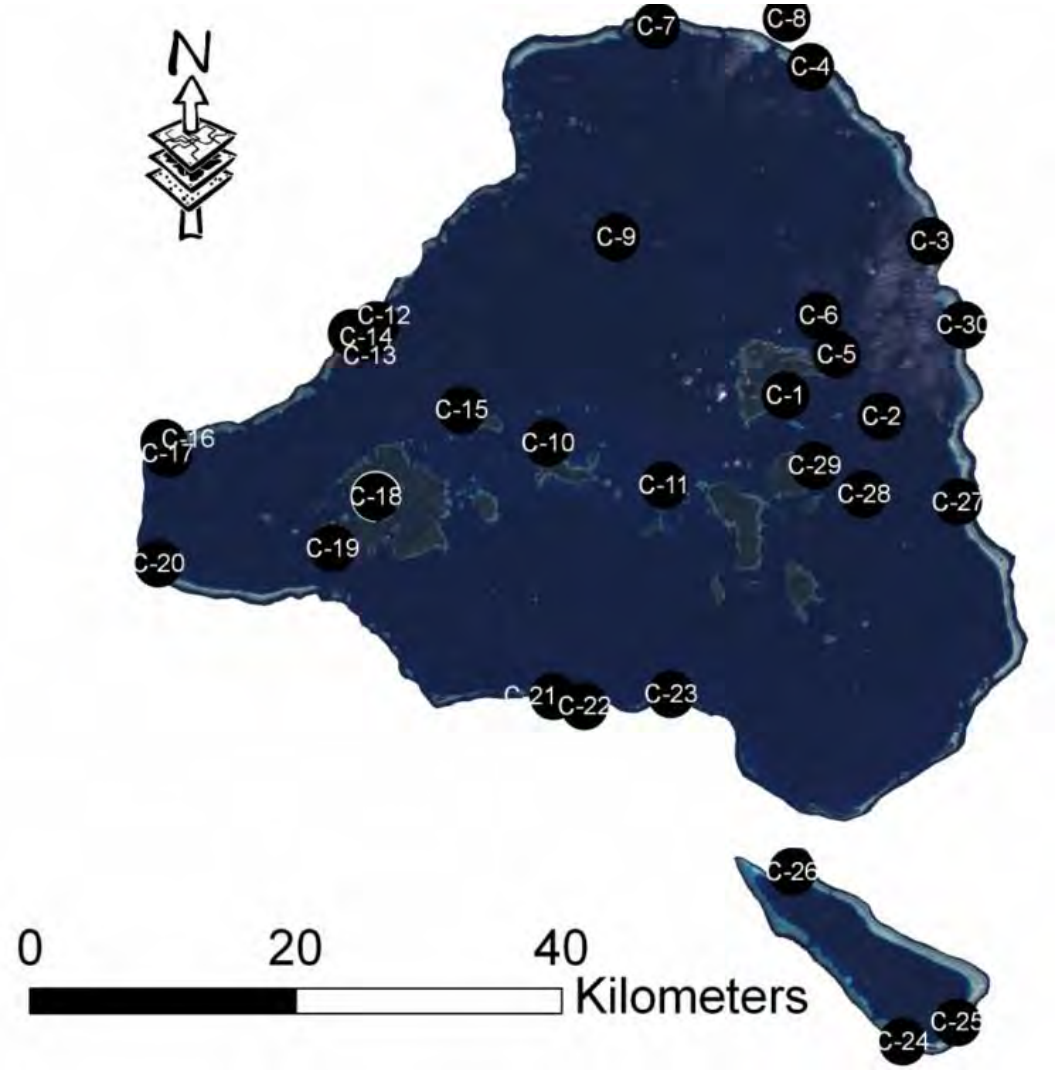


The first thing we notice is that our standard deviations are greatly reduced when examining data at the site level, suggesting our survey goals of detecting change at the site level are better approached. However, they are still higher than desired for many sites. We will touch back on that later.

23. **Remove** the "***StdDev of Sea Cucumber Total***" box from ***Values***.
    a. **Click** and **drag** it back up from where you initially grabbed it.
24. Back on the "PivotChart Filter Pane" window **click** on the drop down menu for "***Depth***" and leave only the 3m depth highlighted.
25. Confirm below.



Three sites seem to stand out as holding relatively high abundances of sea cucumbers for Chuuk, these are C-5, C-15, and C-11. Look at the map below to understand where those sites are.

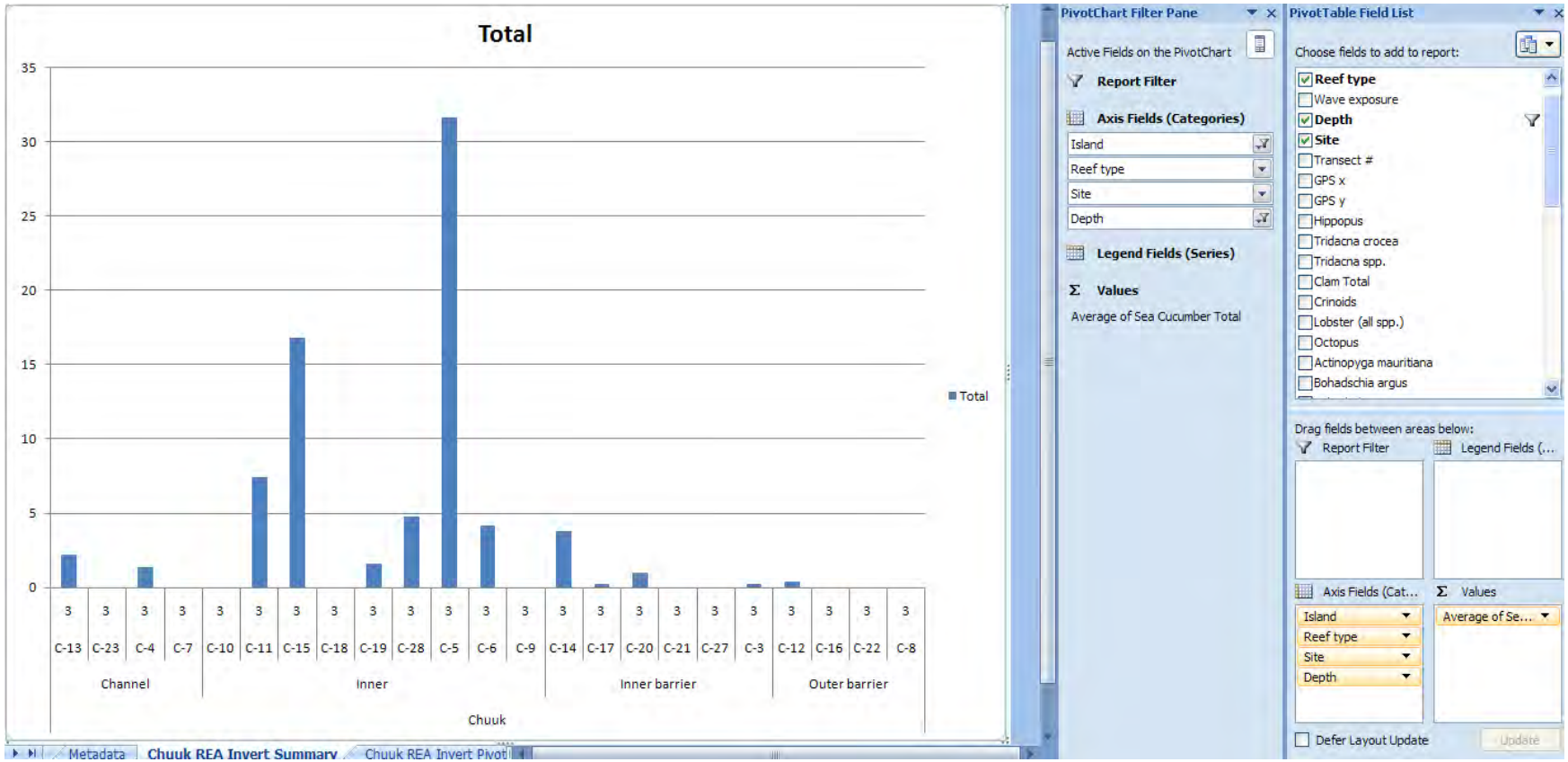


Not surprising the highest abundances were found on Chuuk's inner reefs, adjacent to islands of varying population, land-use, and other physical attributes. However, many similar inner reefs were surveyed, yet why do the abundances vary among them? Let's look closer.

26. From the "***PivotTable Field List***"

a. Click and **drag** the "***Reef type***" box below the "***Island***", but on top of the "***Site***" and "***Depth***" boxes.

b. Confirm below.



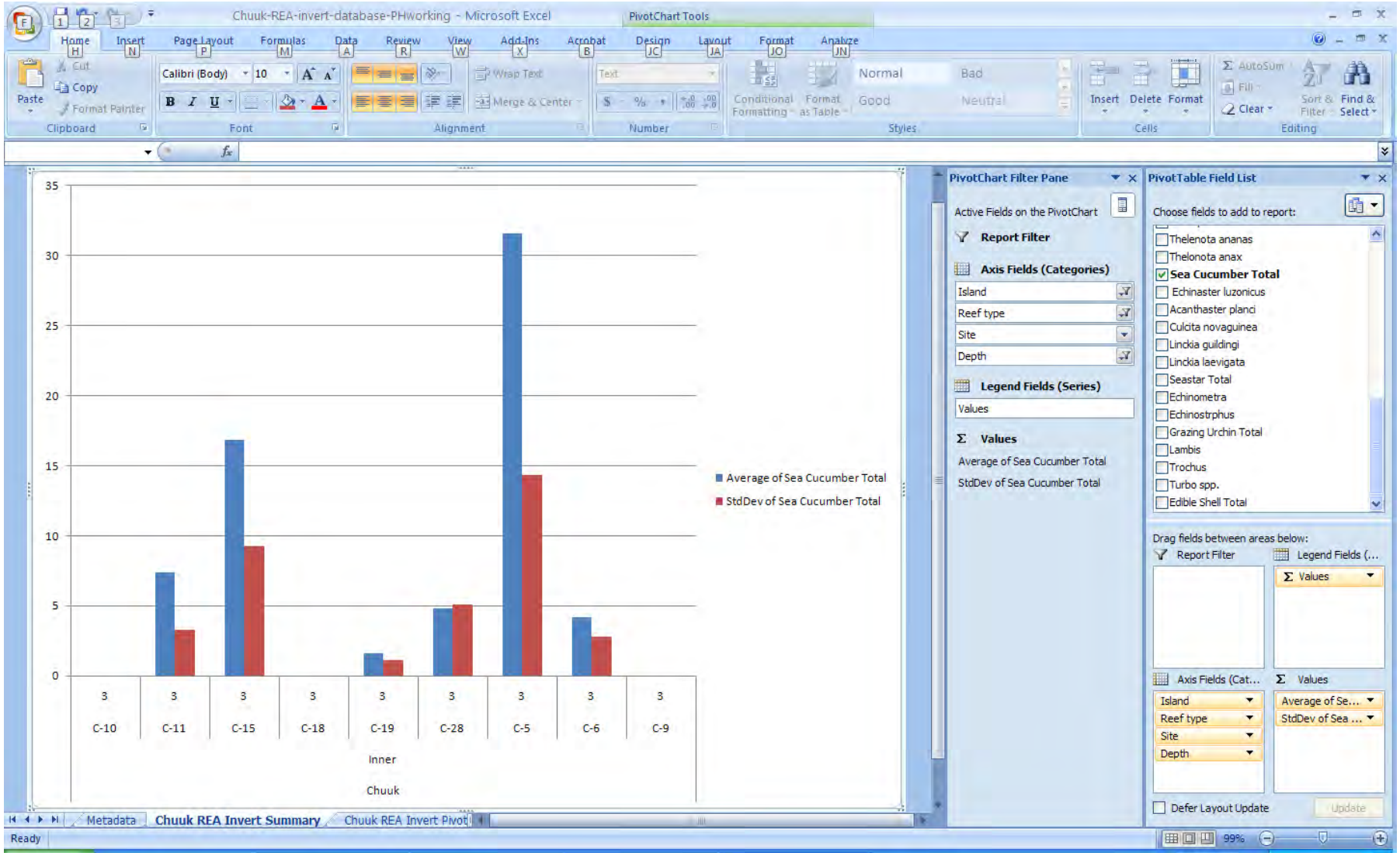
We can now easily see that sea cucumbers are preferably found on the inner reefs as expected, but why is there so much variation among inner reef sites?

27. Go to the ***drop down menu*** for the "***Reef type***" filter pane, **check** only "***inner***".

Nine sites are left on our graph, again you can refer to the map above to understand which inner reefs have the highest abundances of sea cucumber resources. Our final step here will be to investigate the quality of our data collection (i.e., if we re-do the surveys do we have statistical confidence to detect a change, especially at the sites where resources are good).

28. On the "***PivotTable Field List***"

- **a. Click** and **drag** the "***Sea Cucumber Total***" again down to the "***Values***" box, below "***Average of Sea Cucumber Total***".
- **b. Left click** the box one time, **click** on "***Value Field Settings***"
- **c. Set to** "***StdDev***".
- **d.** Confirm below.



We can also look at the 10m depth and find similar patterns, however abundances typically decrease with depth, can you find the site with an exception to this pattern?

In two out of three of the sites where sea cucumbers were most abundant our standard deviation is less than half of our average, or mean. While any coral reef manager would like lower standard deviation bars, this is a satisfactory situation. How do our findings translate to future next steps and potential management actions?
First, one commonly applied rule for management is to protect the locations where good resources exist. It would be insightful to understand why C-5, C-15, and C-11 hold high resource abundances. The two probable causes attributable to patterns are: 1) differing natural environments, or 2) human harvesting trends. This is where scientists and monitoring teams present findings to communities and knowledgeable individuals to learn, and plan for management accordingly. If we can understand what conditions lead to high sea cucumber populations than we can identify and prioritize management actions that should be efficient.
Second, long-term monitoring focused on sea cucumbers for Chuuk seems best focused upon "inner" reefs. People in charge of continued monitoring programs might design, or re-design, annual ecological surveys accordingly. It seems less appropriate to randomly survey all reef habitats in Chuuk, however, like any dataset, the REA data doesn't tell the whole story. For example, the outer reef flats were not surveyed and typically hold high sea cucumber populations, but usually of only a few species.

End of Exercise 2

Exercise 3 – Advanced queries into a large, multivariate dataset to understand ecological patterns pertinent for management actions.

1. **Open** the file "***Pohnpei-MPA-fish-transects***".

Notice there are two sheets that are populated with data and site information.

2. **Click** on the sheet "***Site information***".

This sheet contains a list of all MPA monitoring locations for Pohnpei's program, MPA status, reef-type, indicator fish species, and two coefficients that are used to estimate biomass from length estimates.

3. **Click** on the next sheet "***PNP Fish Database***"

You can see a dataset for Pohnpei's 2006 indicator fish monitoring efforts. First notice the design of the database is different from the Chuuk REA database. Here, each row represents one individual fish on any particular transect, at any particular site. With the Chuuk REA data each row represented one transect.

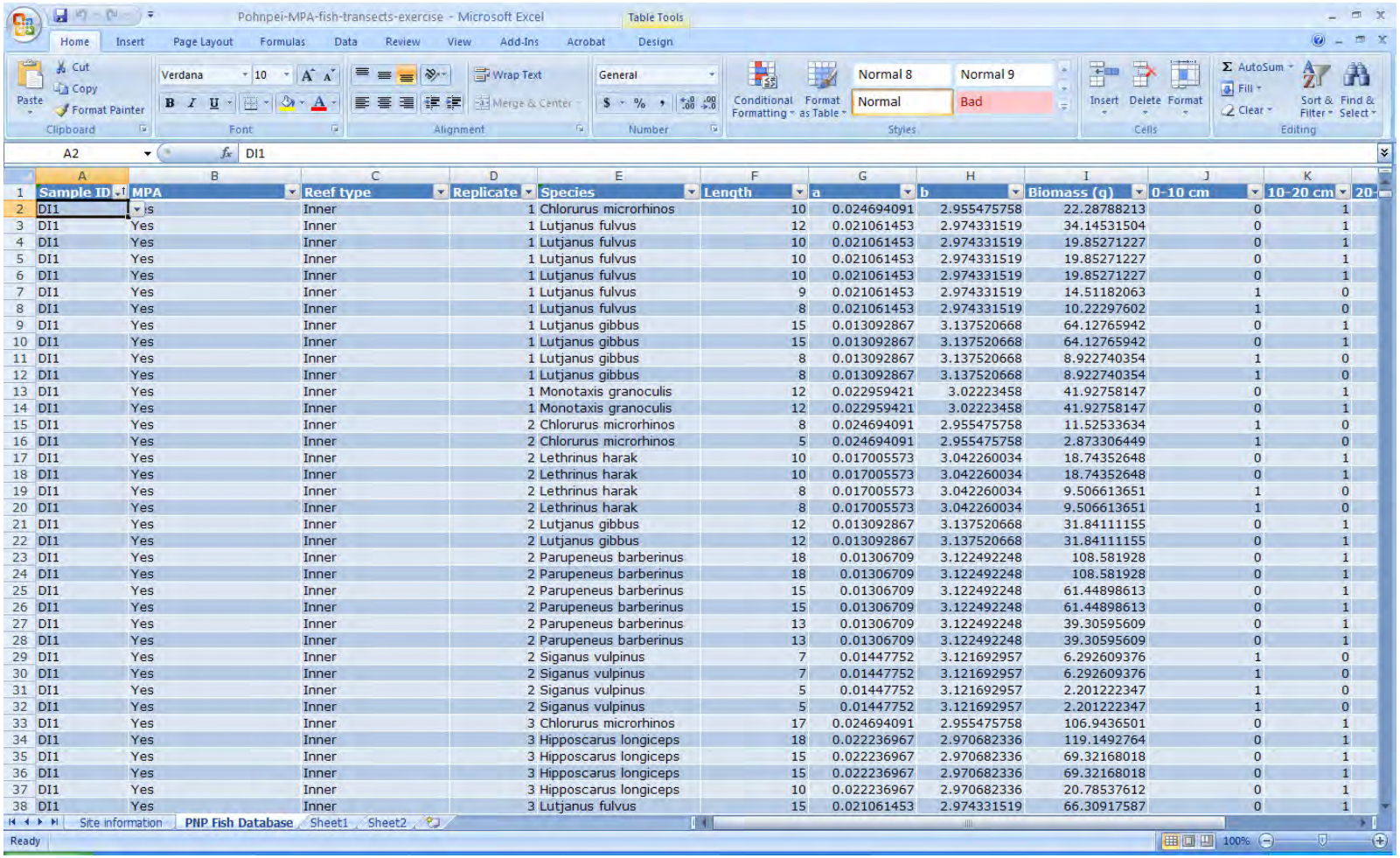
Take time to notice the column headings and how the drop down menu and lookup functions were created.

4. Do this by **clicking** in ***cells A2 across***, and understand how each function works.

The formula for fish biomass comes from published studies and each species coefficients comes from a website called "FishBase", a global initiative to improve our understanding and science surrounding fish and fisheries (www.fishbase.org).

We are going to be manipulating this database to understand trends in MPA success. In the case of any master database, no data queries or graphing should be conducted using the same file as the original database.

5. First do a **"save as"** and **name** the file **"*Pohnpei-MPA-fish-transects-exercise*",** or any other name of your choosing.



Pohnpei-MPA-fish-transects-exercise - Microsoft Excel

Sample ID	MPA	Reef type	Replicate	Species	Length	a	b	Biomass (g)	0-10 cm	10-20 cm	20-
DI1	Yes	Inner	1	Chlorurus microrhinos	10	0.024694091	2.955475758	22.28788213	0	1	
DI1	Yes	Inner	1	Lutjanus fulvus	12	0.021061453	2.974331519	34.14531504	0	1	
DI1	Yes	Inner	1	Lutjanus fulvus	10	0.021061453	2.974331519	19.85271227	0	1	
DI1	Yes	Inner	1	Lutjanus fulvus	10	0.021061453	2.974331519	19.85271227	0	1	
DI1	Yes	Inner	1	Lutjanus fulvus	10	0.021061453	2.974331519	19.85271227	0	1	
DI1	Yes	Inner	1	Lutjanus fulvus	9	0.021061453	2.974331519	14.51182063	1	0	
DI1	Yes	Inner	1	Lutjanus fulvus	8	0.021061453	2.974331519	10.22297602	1	0	
DI1	Yes	Inner	1	Lutjanus gibbus	15	0.013092867	3.137520668	64.12765942	0	1	
DI1	Yes	Inner	1	Lutjanus gibbus	15	0.013092867	3.137520668	64.12765942	0	1	
DI1	Yes	Inner	1	Lutjanus gibbus	8	0.013092867	3.137520668	8.922740354	1	0	
DI1	Yes	Inner	1	Lutjanus gibbus	8	0.013092867	3.137520668	8.922740354	1	0	
DI1	Yes	Inner	1	Monotaxis granoculis	12	0.022959421	3.02223458	41.92758147	0	1	
DI1	Yes	Inner	1	Monotaxis granoculis	12	0.022959421	3.02223458	41.92758147	0	1	
DI1	Yes	Inner	2	Chlorurus microrhinos	8	0.024694091	2.955475758	11.52533634	1	0	
DI1	Yes	Inner	2	Chlorurus microrhinos	5	0.024694091	2.955475758	2.873306449	1	0	
DI1	Yes	Inner	2	Lethrinus harak	10	0.017005573	3.042260034	18.74352648	0	1	
DI1	Yes	Inner	2	Lethrinus harak	10	0.017005573	3.042260034	18.74352648	0	1	
DI1	Yes	Inner	2	Lethrinus harak	8	0.017005573	3.042260034	9.506613651	1	0	
DI1	Yes	Inner	2	Lethrinus harak	8	0.017005573	3.042260034	9.506613651	1	0	
DI1	Yes	Inner	2	Lutjanus gibbus	12	0.013092867	3.137520668	31.84111155	0	1	
DI1	Yes	Inner	2	Lutjanus gibbus	12	0.013092867	3.137520668	31.84111155	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	18	0.01306709	3.122492248	108.581928	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	18	0.01306709	3.122492248	108.581928	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	15	0.01306709	3.122492248	61.44898613	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	15	0.01306709	3.122492248	61.44898613	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	13	0.01306709	3.122492248	39.30595609	0	1	
DI1	Yes	Inner	2	Parupeneus barberinus	13	0.01306709	3.122492248	39.30595609	0	1	
DI1	Yes	Inner	2	Siganus vulpinus	7	0.01447752	3.121692957	6.292609376	1	0	
DI1	Yes	Inner	2	Siganus vulpinus	7	0.01447752	3.121692957	6.292609376	1	0	
DI1	Yes	Inner	2	Siganus vulpinus	5	0.01447752	3.121692957	2.201222347	1	0	
DI1	Yes	Inner	2	Siganus vulpinus	5	0.01447752	3.121692957	2.201222347	1	0	
DI1	Yes	Inner	3	Chlorurus microrhinos	17	0.024694091	2.955475758	106.9436501	0	1	
DI1	Yes	Inner	3	Hipposcarus longiceps	18	0.022236967	2.970682336	119.1492764	0	1	
DI1	Yes	Inner	3	Hipposcarus longiceps	15	0.022236967	2.970682336	69.32168018	0	1	
DI1	Yes	Inner	3	Hipposcarus longiceps	15	0.022236967	2.970682336	69.32168018	0	1	
DI1	Yes	Inner	3	Hipposcarus longiceps	10	0.022236967	2.970682336	20.78537612	0	1	
DI1	Yes	Inner	3	Lutjanus fulvus	15	0.021061453	2.974331519	66.30917587	0	1	

Now we are ready to begin our query and investigation. Logically, we'll start by asking the most general questions, and get more specific as we learn.

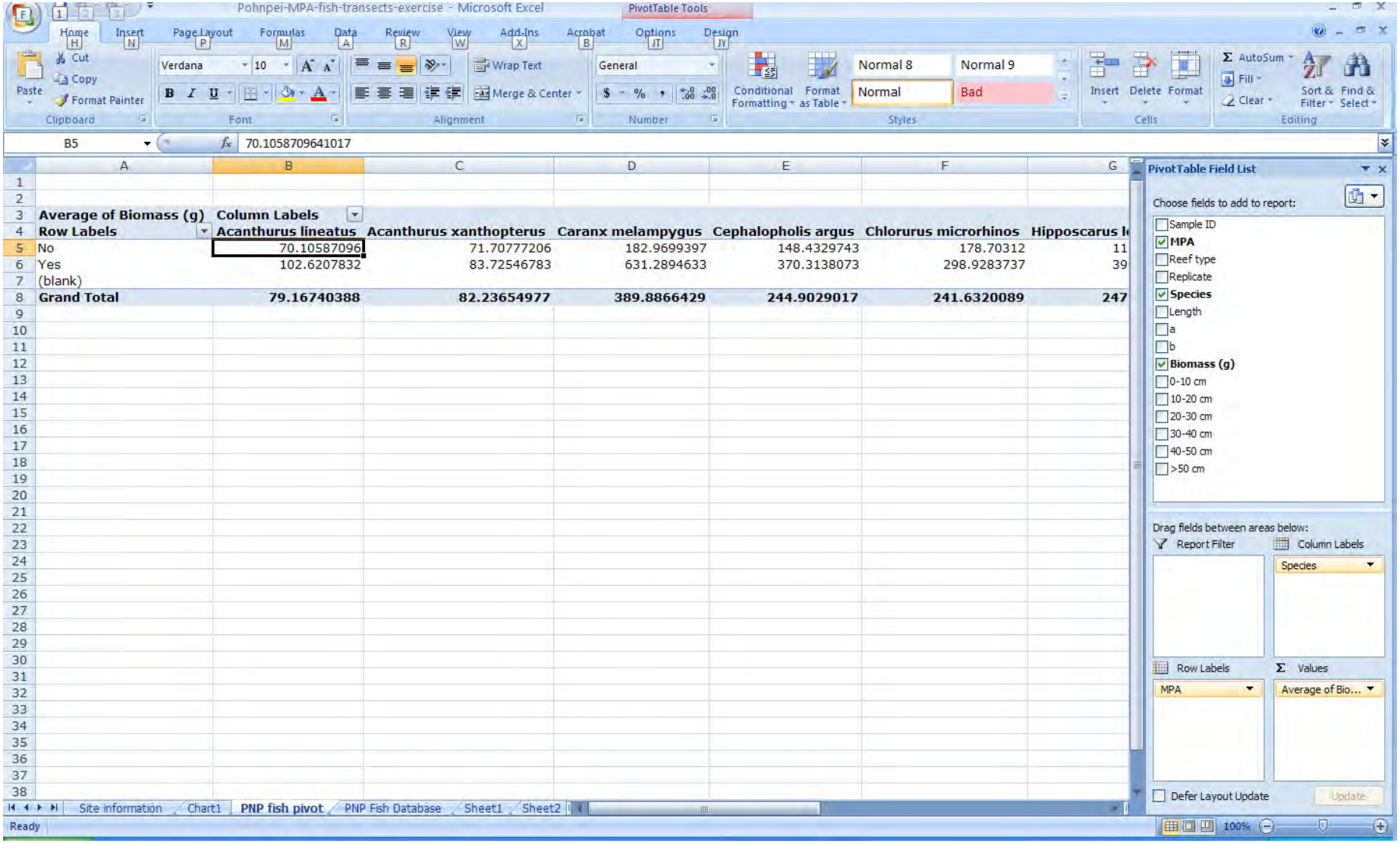
6. **Highlight** all of the data and again **insert** a ***Pivot Table*** like before.

   a. **Change** the name of the sheet to "***PNP fish pivot***"

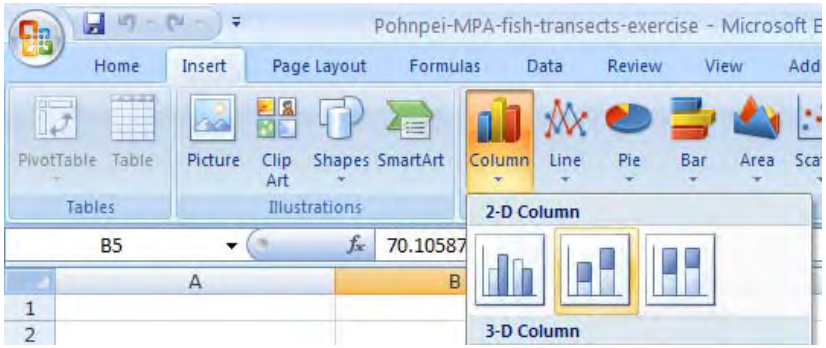
b. Click ok in the dialog box.

We will first take a look at all MPA's grouped together, not yet taking into account statistical sampling concerns like standard deviations and confidence intervals surrounding our data.

7. Click and **drag** the "***MPA***" box and put in under "***Row Labels***".
   a. Put "***Species***" under "***Column labels***"
   b. Put "***Biomass***" under "***Values***".
   c. Left click the "***Count of Biomass***" box once and **change** the field settings to "***average***".
   d. Confirm below.



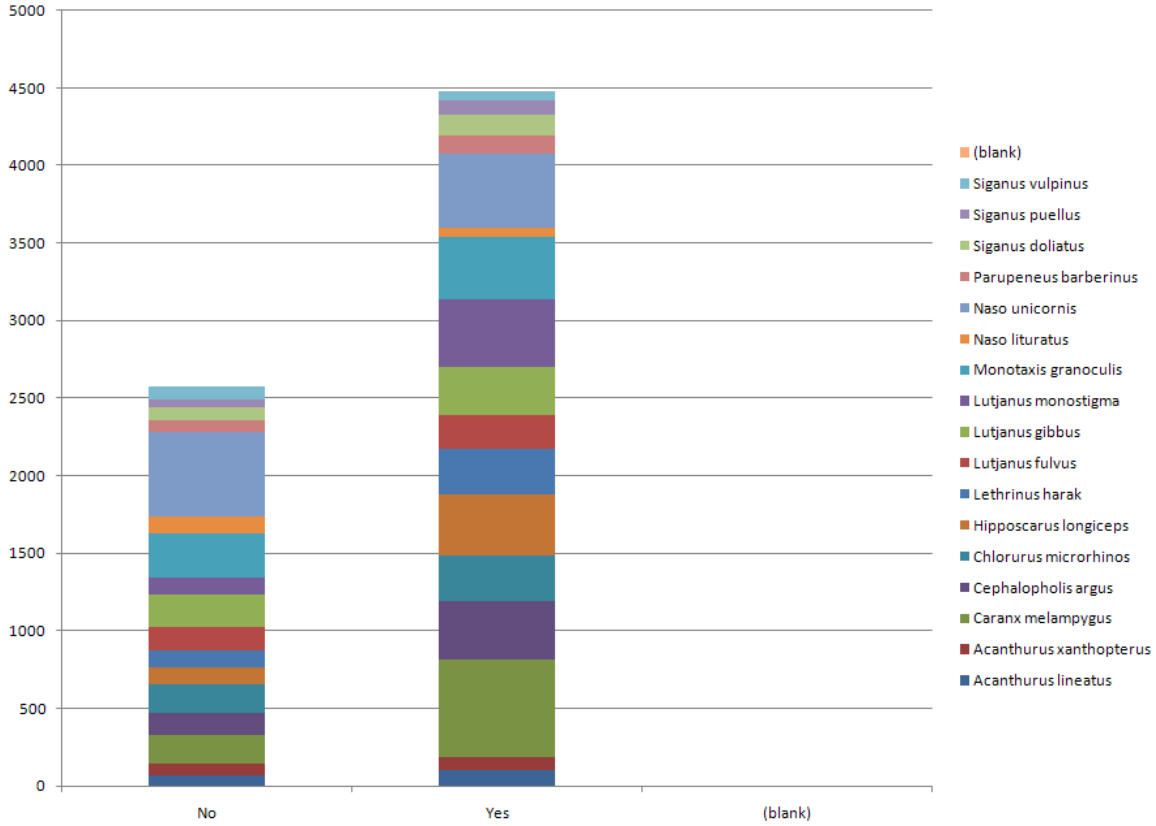
8. Go to the "***Insert***" tab off the main menu of Excel and **insert** a column chart,
   a. **Choose** the ***stacked column chart*** one showing cumulative data summaries.



b. **Right click** in the chart area and **move** this chart to its own sheet.

c. **Name** the sheet "***PNP fish pivot chart***".

d. Confirm below.

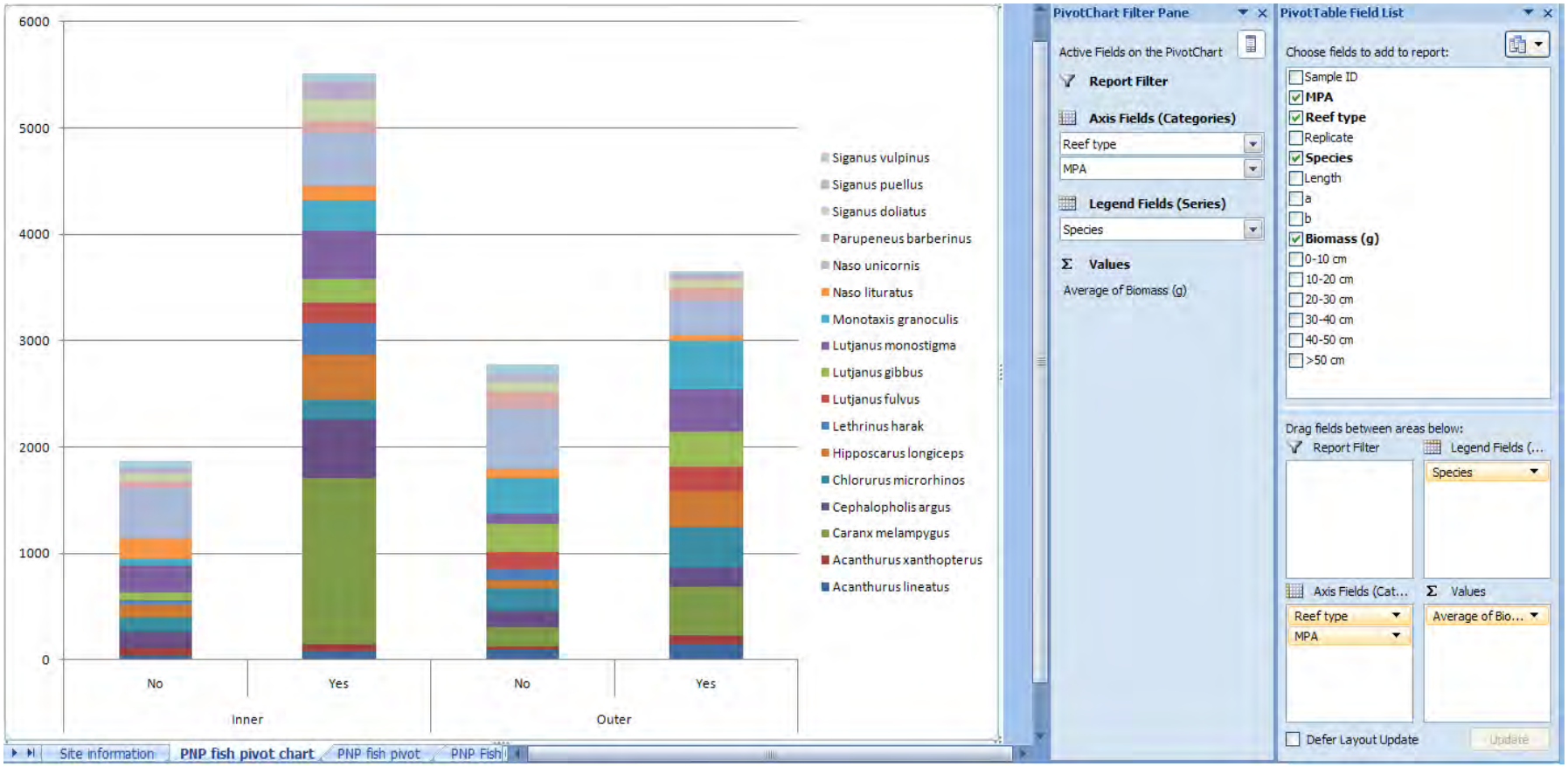


9. **Click** on the "***MPA***" drop down menu in the PivotChart Filter Pane,

a. **Uncheck** the "***blank***" box if it happens to be selected, if not you don't need to do anything.

This initial chart seems like positive news, on average there is a greater biomass of just about every indicator species inside of the MPA's compared with outside. However, there is a lot more to consider before coming to that conclusion so we should continue our investigation.

10. Click and drag the "***Reef type***" box and **drag** it into the ***fields box***, putting it above "***MPA***".



Now we can clearly see that inner reef MPA's are protecting a much larger proportion of the biomass as compared with outer reefs. Specifically, Caranx melampygus (jack) and Hipposcarus longiceps (parrotfish) are two fish that seem to be influential drivers of this trend. Are these differences in success based upon reef type due to proximity of human populations that help maintain and enforce the MPA? Are they due to natural differences in habitat types, whereby outer reefs are harder to access so differences are less

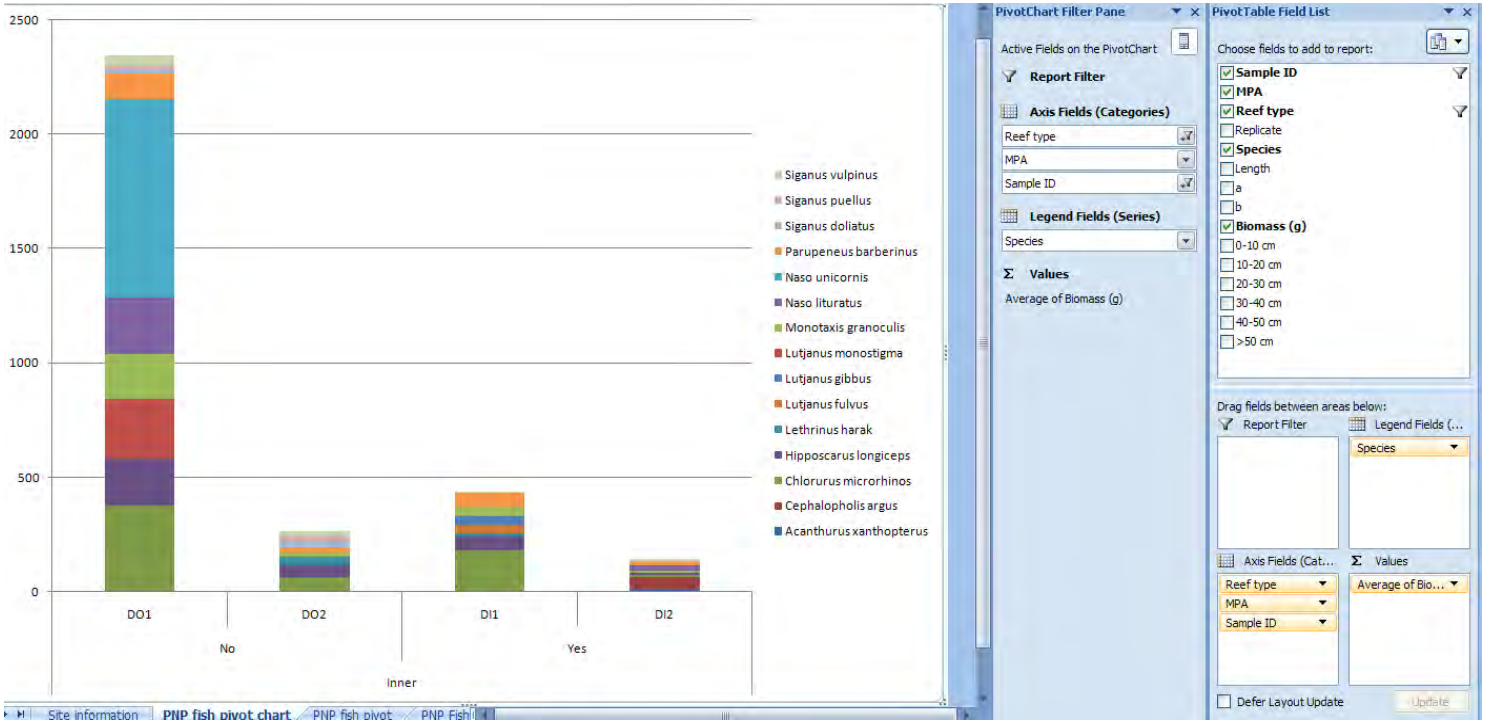
pronounced? We must be clear that we can't answer these questions with our existing data, but we can continue to learn about patterns so we know how to most efficiently learn about cause.

Let's focus more on understanding patterns for the "inner" reefs as they seem most influential.

11. On the PivotChart Filter Pane, **click** the drop down menu next to "***Reef type***", and leave only inner reefs checked.

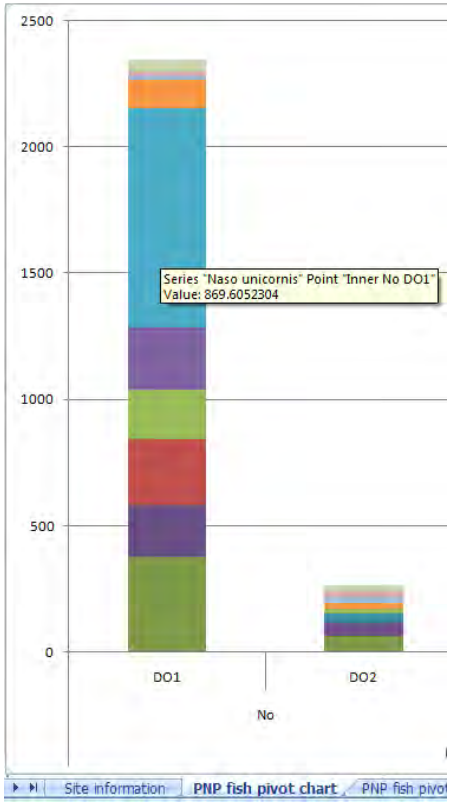
- **a. Click** the "***SampleID***" box and **drag** it below "***MPA***" in the ***Axis Fields box***.
- **b.** Go back to the PivotChart Filter Pane and go to the drop down menu for "***SampleID***".
- **c. Check** only the boxes for "***DI1***", "***DI2***", "***DO1***", and "***DO2***".

This means that we are going to look at data from the MPA with nickname "D" and the sites surveyed "I" inside and "O" outside the MPA. The "1" and "2" refer to site replicates within which 5 x 50m transects were surveyed. Indeed, a nice survey design, methods, and dataset. Confirm below.



These results contradict our earlier finding of success for MPA's in general. For this MPA we see there appear to be more fish outside the MPA compared with inside. Check to agree that these trends are especially pronounced for Chlorurus microrhinos and Naso unicornis at the "DO1" site, outside the MPA.

Note: You can hover over any part of the data bar and Excel should automatically tell you what fish species each color represents. Confirm below if it is not clear.



Let's look at the next MPA.

12. Go back to the PivotChart Filter Pane and

- **a. Go to** the drop down menu for "***SampleID***".
- ***b.*** **Check** only the boxes for "***KI1***", "***KI2***", "***KO1***", and "***KO2***". *This means that we are going to look at data from the MPA with nickname "K" now.*

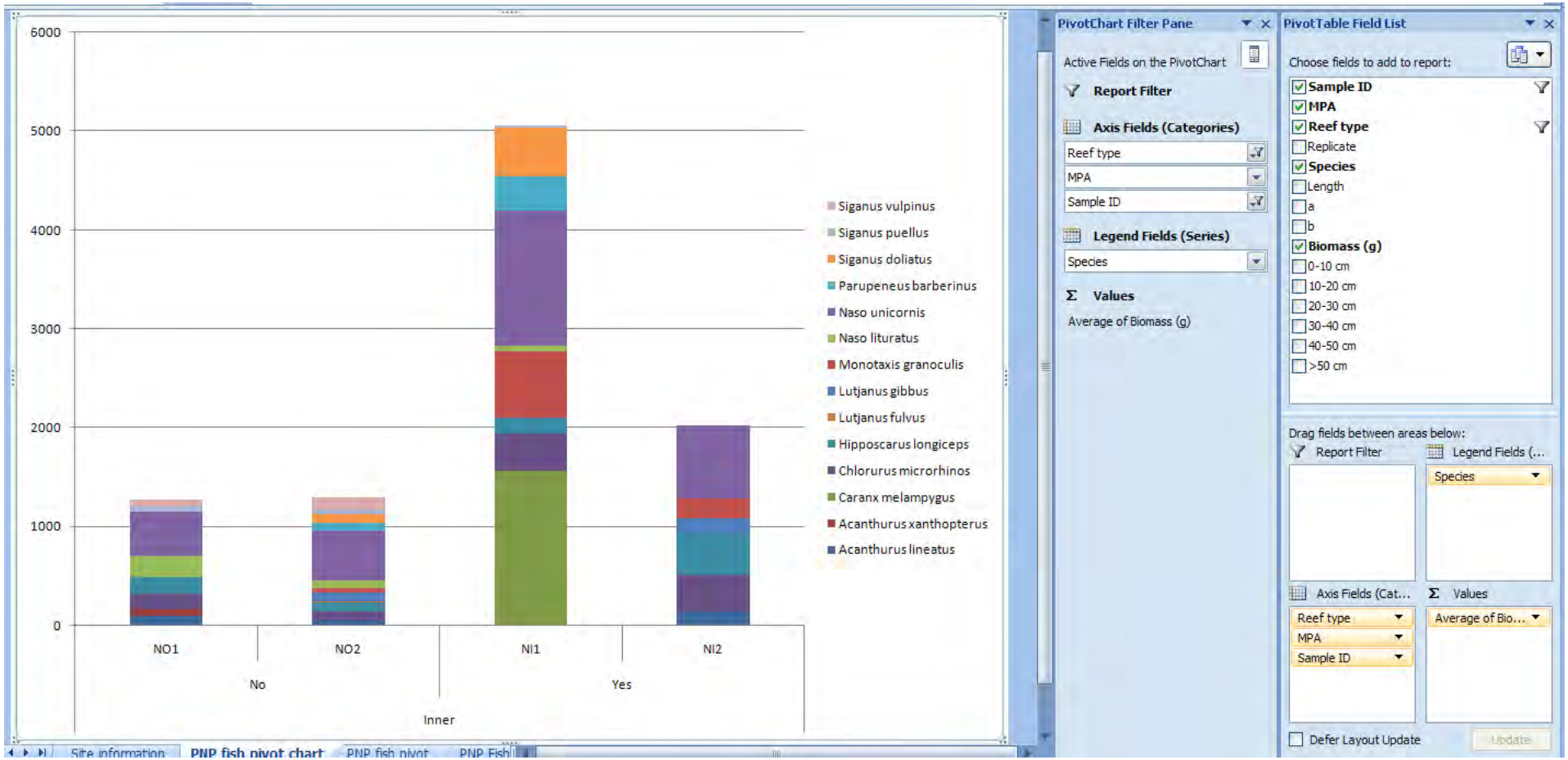
The results again clearly show no substantial benefits of the "K" MPA site.

Let's continue because we know the overall trends suggested MPA's were working on the whole.

13. Go back to the ***PivotChart Filter Pane***

- **a. Go to** the drop down menu for "***SampleID***".
- **b. Check** only the boxes for "***LI1***", "***LI2***", "***LO1***", and "***LO2***".

Now we can easily see the perceived success of this MPA compared with others.
You can do the same examinations for MPA's "M" and "N". You can confirm below for MPA N".



We have learned a great deal from our investigations thus far. First, for inner reefs, MPA's "D" and "K" do not seem successful as compared with all other three. Second, by far, the most success seems to be found at MPA "L". Third, although team Pohnpei monitors 16 indicator fish, trends are most influentially delineated by only a few fish. Namely, these are Chlorurus microrhinos, Hipposcarus longiceps, Caranx melampygus, Naso unicornis, and maybe one or two others. This is understandable because these are relatively large fish that make up a high proportion of Pohnpei's fish market catch. It is very interesting to learn that fewer fish may be able to serve as statistically useful indicators for MPA success, and these are common with local names that are well known.

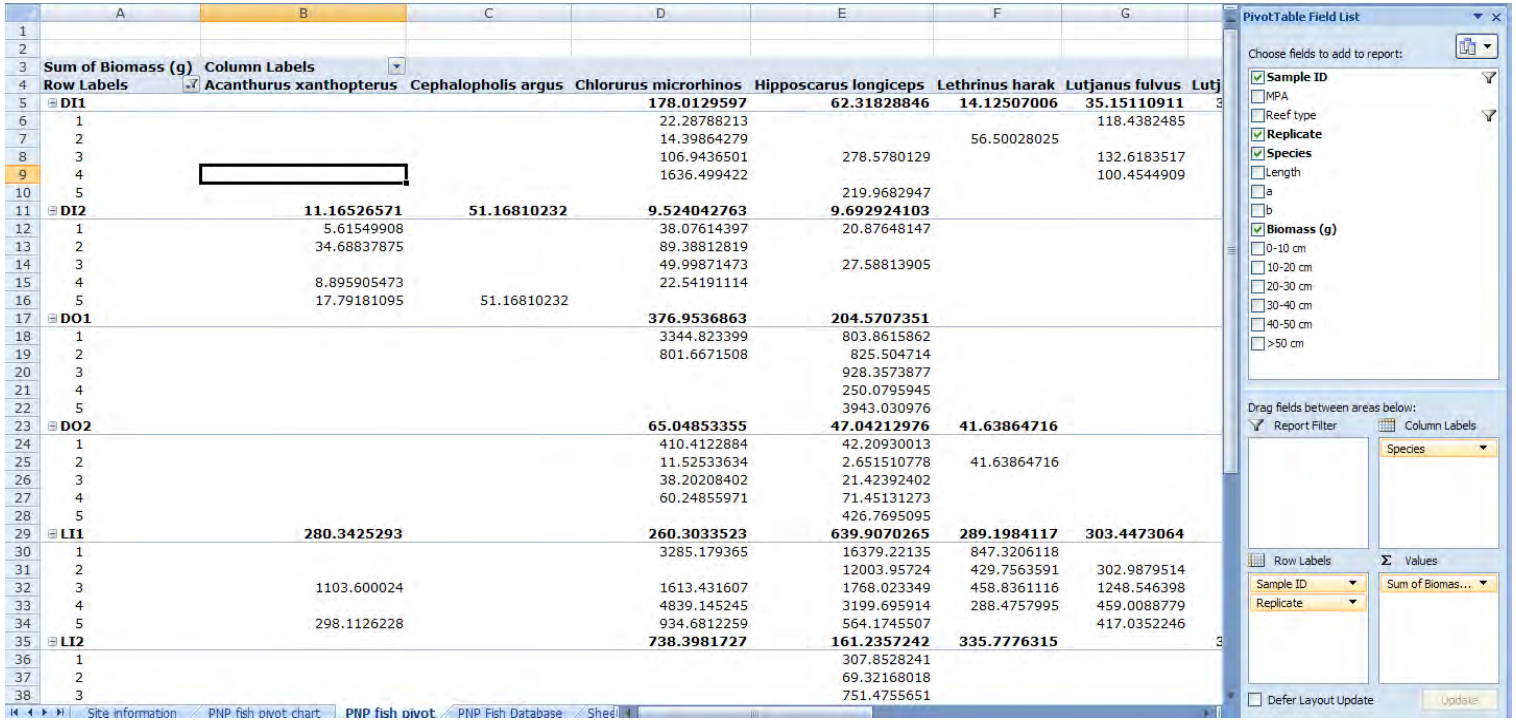
End of Exercise 3, save the file, and keep it open. This same file will be used for Exercise 4.

Exercise 4 – Beyond examining trends. Reformatting an existing database to understand statistical aspects of the data.

While we have successfully visualized trends regarding fish assemblages from Pohnpei's MPA dataset in Exercise 3, will now take a look at the statistical confidence of these data, as we have yet to view any error bars that describe consistencies among transects and sites. Because the original database was generated by placing each individual fish measurement in its own row with lots of metadata, we will need to re-format the dataset to generate summaries at the transect-level. Recall, the transect is our unit of replication within each site. It is good to understand the functional differences for different database formats, take a moment to reflect.

Programs like Excel make it relatively easy to switch formats in a short time frame.

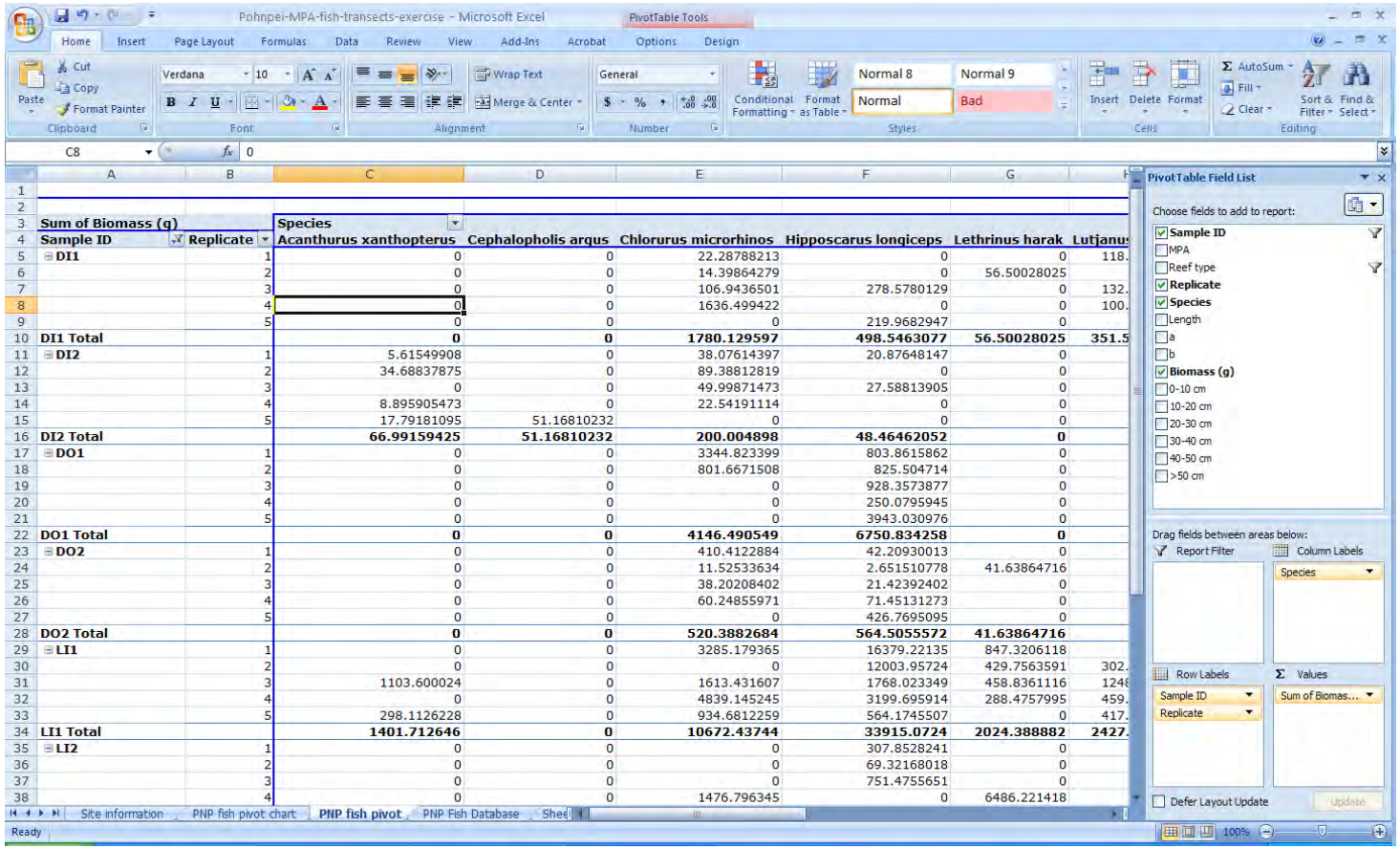
1. First, go back to our "***PNP fish pivot***" worksheet.
2. Under "***Row Labels***" **click** and **drag** the boxes for "***MPA***" and "***Reef type***" out.
3. **Click** and **drag** "***Replicate***" and put it under "***SampleID***".
   a. Under values, **left click** once on "***Average of Biomass***", and **change** the attribute field to "***Sum***".
   b. Confirm below.



Sum of Biomass (g)	Column Labels						
Row Labels	Acanthurus xanthopterus	Cephalopholis argus	Chlorurus microrhinos	Hipposcarus longiceps	Lethrinus harak	Lutjanus fulvus	Lutj
DI1			**178.0129597**	**62.31828846**	**14.12507006**	**35.15110911**	3
1			22.28788213			118.4382485	
2			14.39864279		56.50028025		
3			106.9436501	278.5780129		132.6183517	
4			1636.499422			100.4544909	
5				219.9682947			
DI2	**11.16526571**	**51.16810232**	**9.524042763**	**9.692924103**			
1	5.61549908		38.07614397	20.87648147			
2	34.68837875		89.38812819				
3			49.99871473	27.58813905			
4	8.895905473		22.54191114				
5	17.79181095	51.16810232					
DO1			**376.9536863**	**204.5707351**			
1			3344.823399	803.8615862			
2			801.6671508	825.504714			
3				928.3573877			
4				250.0795945			
5				3943.030976			
DO2			**65.04853355**	**47.04212976**	**41.63864716**		
1			410.4122884	42.20930013			
2			11.52533634	2.651510778	41.63864716		
3			38.20208402	21.42392402			
4			60.24855971	71.45131273			
5				426.7695095			
LI1	**280.3425293**		**260.3033523**	**639.9070265**	**289.1984117**	**303.4473064**	
1			3285.179365	16379.22135	847.3206118		
2				12003.95724	429.7563591	302.9879514	
3	1103.600024		1613.431607	1768.023349	458.8361116	1248.546398	
4			4839.145245	3199.695914	288.4757995	459.0088779	
5	298.1126228		934.6812259	564.1745507		417.0352246	
LI2			**738.3981727**	**161.2357242**	**335.7776315**		3
1				307.8528241			
2				69.32168018			
3				751.4755651			

Now we have a spreadsheet with each replicate transect as a row, and a total amount of fish biomass recorded on each transect (hence the sum instead of average function). This is exactly what we need to examine transect-level replication, total sums of biomass for each species along each transect.

4. **Right click** anywhere in the table and go to "***Pivot Table Options***".
   a. Make sure the "***Layout&Format***" tab is selected and **put** a "***0***" in the box next to "***For empty cells show***:".
5. **Click** on the "***Totals&Filters***" tab.
   a. **Uncheck** the boxes for "***Show grand totals***", both of them.
6. **Click** on the "***Display***" tab.
   a. **Check** the box that says "***Classic Pivot Table layout***"
7. **Click** OK to close the dialog box.
8. Confirm below.



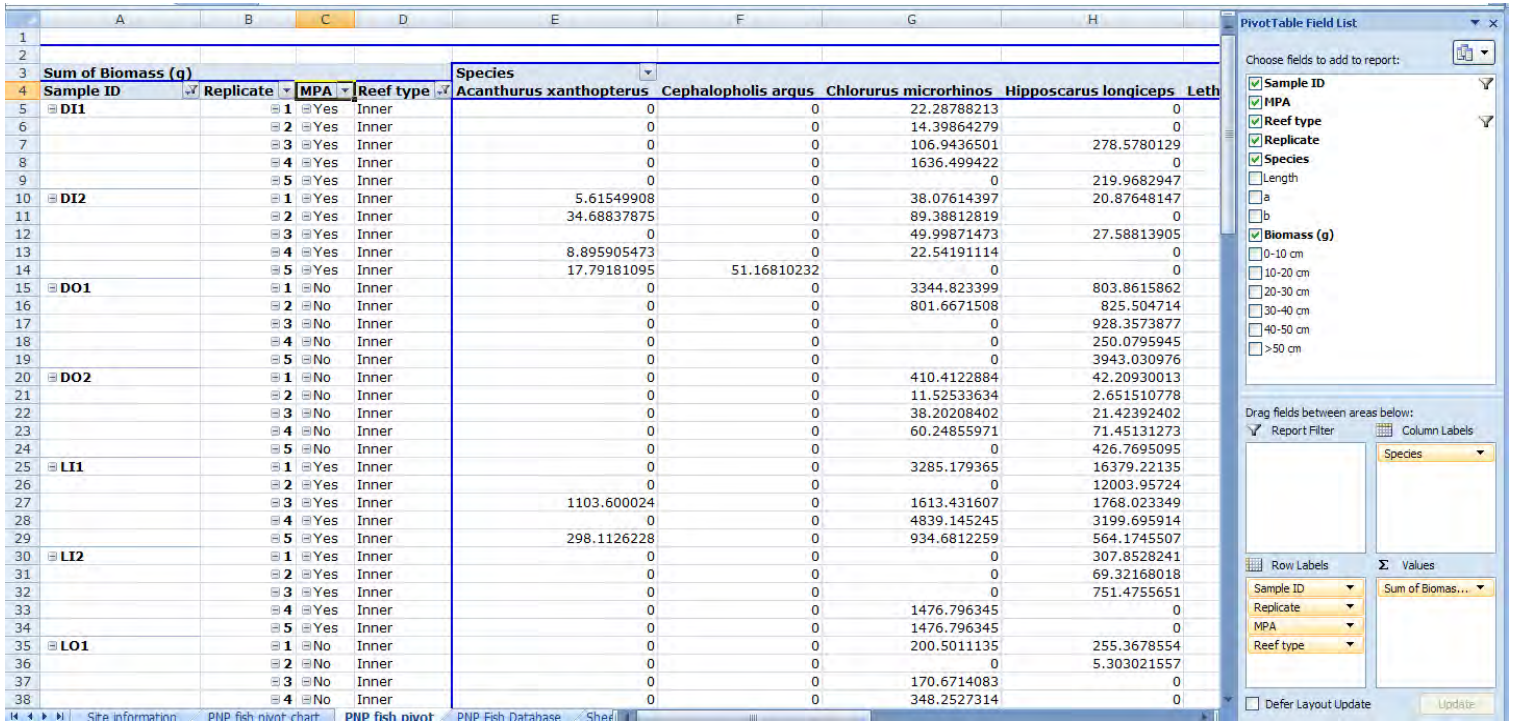
Just a few more steps and then we will have re-created a new database for our needs.

9. **Click** on cell ***A4***, on the pivot table.
   a. **Right click** and choose "***Field Settings***"
   b. Under "***Subtotals***" **click** none.
   c. **Click** OK to close the dialog box.

Now we want to re-add the "MPA" and "Reef type" information in our table.

10. **Click** and **drag** these boxes and **put** them under "***Replicate***".
11. **Click** on cell ***B4***, again choose "***Field Settings***"
    a. Under "***Subtotals***" **click** none.
    b. **Click** OK to close the dialog box.

12. **Click** on cell ***C4***, again choose "***Field Settings***",
    a. Under "***Subtotals***" **click** none.
    b. **Click** OK to close the dialog box.
13. Confirm below.



Sum of Biomass (g)				Species				
Sample ID	Replicate	MPA	Reef type	Acanthurus xanthopterus	Cephalopholis argus	Chlorurus microrhinos	Hipposcarus longiceps	Leth
DI1	1	Yes	Inner	0	0	22.28788213	0	
	2	Yes	Inner	0	0	14.39864279	0	
	3	Yes	Inner	0	0	106.9436501	278.5780129	
	4	Yes	Inner	0	0	1636.499422	0	
	5	Yes	Inner	0	0	0	219.9682947	
DI2	1	Yes	Inner	5.61549908	0	38.07614397	20.87648147	
	2	Yes	Inner	34.68837875	0	89.38812819	0	
	3	Yes	Inner	0	0	49.99871473	27.58813905	
	4	Yes	Inner	8.895905473	0	22.54191114	0	
	5	Yes	Inner	17.79181095	51.16810232	0	0	
DO1	1	No	Inner	0	0	3344.823399	803.8615862	
	2	No	Inner	0	0	801.6671508	825.504714	
	3	No	Inner	0	0	0	928.3573877	
	4	No	Inner	0	0	0	250.0795945	
	5	No	Inner	0	0	0	3943.030976	
DO2	1	No	Inner	0	0	410.4122884	42.20930013	
	2	No	Inner	0	0	11.52533634	2.651510778	
	3	No	Inner	0	0	38.20208402	21.42392402	
	4	No	Inner	0	0	60.24855971	71.45131273	
	5	No	Inner	0	0	0	426.7695095	
LI1	1	Yes	Inner	0	0	3285.179365	16379.22135	
	2	Yes	Inner	0	0	0	12003.95724	
	3	Yes	Inner	1103.600024	0	1613.431607	1768.023349	
	4	Yes	Inner	0	0	4839.145245	3199.695914	
	5	Yes	Inner	298.1126228	0	934.6812259	564.1745507	
LI2	1	Yes	Inner	0	0	0	307.8528241	
	2	Yes	Inner	0	0	0	69.32168018	
	3	Yes	Inner	0	0	0	751.4755651	
	4	Yes	Inner	0	0	1476.796345	0	
	5	Yes	Inner	0	0	1476.796345	0	
LO1	1	No	Inner	0	0	200.5011135	255.3678554	
	2	No	Inner	0	0	0	5.303021557	
	3	No	Inner	0	0	170.6714083	0	
	4	No	Inner	0	0	348.2527314	0	

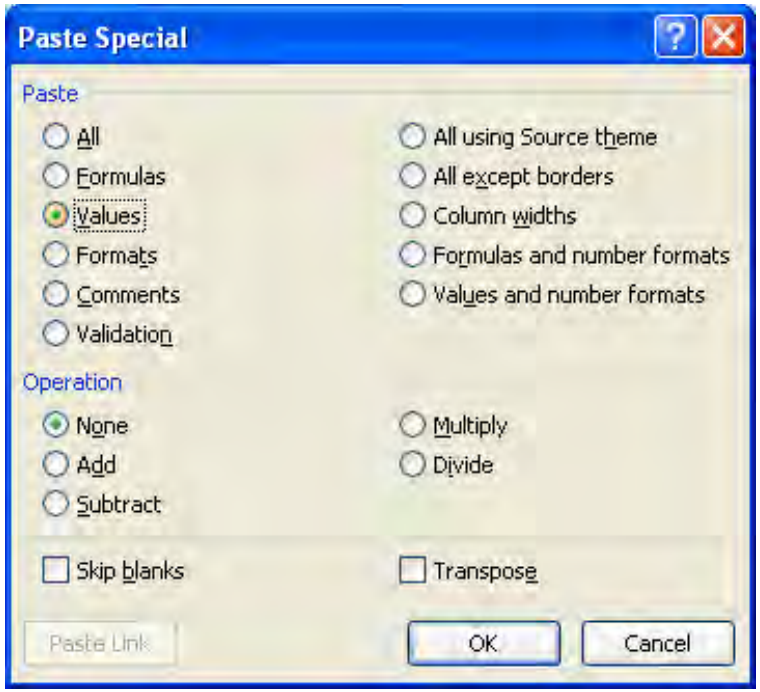
Note: This is the layout of the table we want to export for further investigation.
Note: It's a good idea to save your work at this point.

14. **Check** the drop down menus for "***SampleID***", "***Replicate***", "***MPA***", and "***Reef type***" (*these are cells **A4**, **B4**, **C4**, and **D4***)

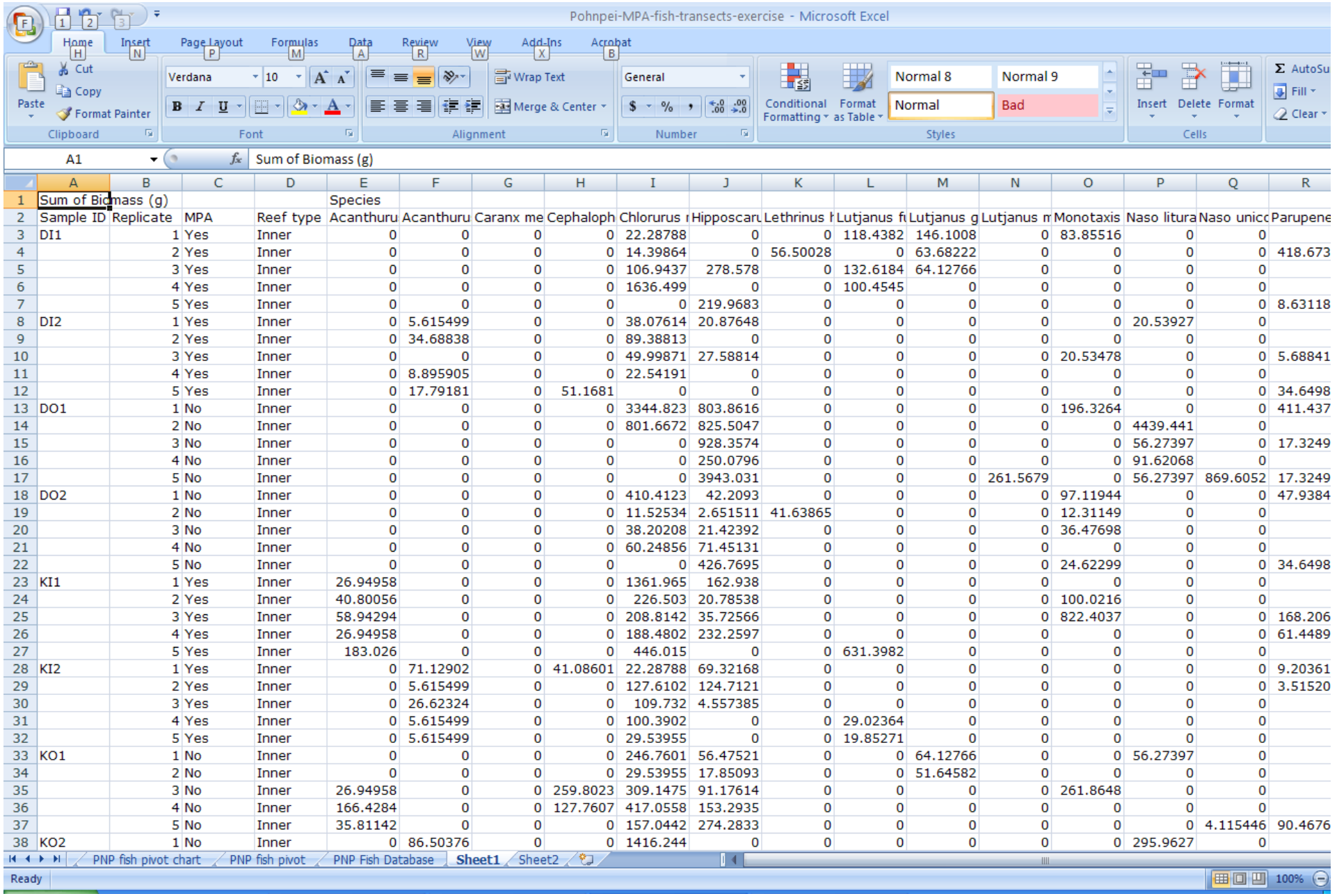
***Note**: Make sure no filters are on and all boxes have a green check mark.*

15. **Click** anywhere inside the pivot table
    a. **Press** the ***Ctrl + A*** buttons on the keyboard to select all the data.
    b. **Right click** again and **select** "***copy***".
16. **Click** the Excel worksheet named "***Sheet 1***".
    a. **Click** in cell ***A1***.

b. **Right click** and **select** "***Paste Special***"
c. **Select** "***Values***"
d. **Click** OK.
e. Confirm below.



You should now have a new formatted sheet. Confirm below.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Sum of Biomass (g)				Species													
2	Sample ID	Replicate	MPA	Reef type	Acanthuru	Acanthuru	Caranx me	Cephaloph	Chlorurus r	Hipposcaru	Lethrinus h	Lutjanus fu	Lutjanus g	Lutjanus m	Monotaxis	Naso litura	Naso unicc	Parupene
3	DI1	1	Yes	Inner	0	0	0	0	22.28788	0	0	118.4382	146.1008	0	83.85516	0	0	
4		2	Yes	Inner	0	0	0	0	14.39864	0	56.50028	0	63.68222	0	0	0	0	418.673
5		3	Yes	Inner	0	0	0	0	106.9437	278.578	0	132.6184	64.12766	0	0	0	0	
6		4	Yes	Inner	0	0	0	0	1636.499	0	0	100.4545	0	0	0	0	0	
7		5	Yes	Inner	0	0	0	0	0	219.9683	0	0	0	0	0	0	0	8.63118
8	DI2	1	Yes	Inner	0	5.615499	0	0	38.07614	20.87648	0	0	0	0	0	20.53927	0	
9		2	Yes	Inner	0	34.68838	0	0	89.38813	0	0	0	0	0	0	0	0	
10		3	Yes	Inner	0	0	0	0	49.99871	27.58814	0	0	0	0	20.53478	0	0	5.68841
11		4	Yes	Inner	0	8.895905	0	0	22.54191	0	0	0	0	0	0	0	0	
12		5	Yes	Inner	0	17.79181	0	51.1681	0	0	0	0	0	0	0	0	0	34.6498
13	DO1	1	No	Inner	0	0	0	0	3344.823	803.8616	0	0	0	0	196.3264	0	0	411.437
14		2	No	Inner	0	0	0	0	801.6672	825.5047	0	0	0	0	0	4439.441	0	
15		3	No	Inner	0	0	0	0	0	928.3574	0	0	0	0	0	56.27397	0	17.3249
16		4	No	Inner	0	0	0	0	0	250.0796	0	0	0	0	0	91.62068	0	
17		5	No	Inner	0	0	0	0	0	3943.031	0	0	0	261.5679	0	56.27397	869.6052	17.3249
18	DO2	1	No	Inner	0	0	0	0	410.4123	42.2093	0	0	0	0	97.11944	0	0	47.9384
19		2	No	Inner	0	0	0	0	11.52534	2.651511	41.63865	0	0	0	12.31149	0	0	
20		3	No	Inner	0	0	0	0	38.20208	21.42392	0	0	0	0	36.47698	0	0	
21		4	No	Inner	0	0	0	0	60.24856	71.45131	0	0	0	0	0	0	0	
22		5	No	Inner	0	0	0	0	0	426.7695	0	0	0	0	24.62299	0	0	34.6498
23	KI1	1	Yes	Inner	26.94958	0	0	0	1361.965	162.938	0	0	0	0	0	0	0	
24		2	Yes	Inner	40.80056	0	0	0	226.503	20.78538	0	0	0	0	100.0216	0	0	
25		3	Yes	Inner	58.94294	0	0	0	208.8142	35.72566	0	0	0	0	822.4037	0	0	168.206
26		4	Yes	Inner	26.94958	0	0	0	188.4802	232.2597	0	0	0	0	0	0	0	61.4489
27		5	Yes	Inner	183.026	0	0	0	446.015	0	0	631.3982	0	0	0	0	0	
28	KI2	1	Yes	Inner	0	71.12902	0	41.08601	22.28788	69.32168	0	0	0	0	0	0	0	9.20361
29		2	Yes	Inner	0	5.615499	0	0	127.6102	124.7121	0	0	0	0	0	0	0	3.51520
30		3	Yes	Inner	0	26.62324	0	0	109.732	4.557385	0	0	0	0	0	0	0	
31		4	Yes	Inner	0	5.615499	0	0	100.3902	0	0	29.02364	0	0	0	0	0	
32		5	Yes	Inner	0	5.615499	0	0	29.53955	0	0	19.85271	0	0	0	0	0	
33	KO1	1	No	Inner	0	0	0	0	246.7601	56.47521	0	0	64.12766	0	0	56.27397	0	
34		2	No	Inner	0	0	0	0	29.53955	17.85093	0	0	51.64582	0	0	0	0	
35		3	No	Inner	26.94958	0	0	259.8023	309.1475	91.17614	0	0	0	0	261.8648	0	0	
36		4	No	Inner	166.4284	0	0	127.7607	417.0558	153.2935	0	0	0	0	0	0	0	
37		5	No	Inner	35.81142	0	0	0	157.0442	274.2833	0	0	0	0	0	0	4.115446	90.4676
38	KO2	1	No	Inner	0	86.50376	0	0	1416.244	0	0	0	0	0	0	295.9627	0	

17. **Rename** this sheet from "***Sheet 1***" to "***PNP Fish Data by Transect***".

18. **Delete** "***Row 1***"
    a. **Highlight** the rest of the data (***Ctrl + A***)
19. **Go to** the ***insert tab*** from Excel's main menu, and **choose** "***Table***".
20. **Click** OK
21. Confirm below.

	A	B	C	D	E	F	G	H	I	J	K
1	Sample II	Replicati	MPA	Reef type	Acanthurus lineati	Acanthurus xanthopter	Caranx melampyg	Cephalopholis arg	Chlorurus microrhin	Hipposcarus longice	Lethrinus ha
2	DI1	1	Yes	Inner	0	0	0	0	22.28788213	0	
3		2	Yes	Inner	0	0	0	0	14.39864279	0	56.5002
4		3	Yes	Inner	0	0	0	0	106.9436501	278.5780129	
5		4	Yes	Inner	0	0	0	0	1636.499422	0	
6		5	Yes	Inner	0	0	0	0	0	219.9682947	
7	DI2	1	Yes	Inner	0	5.61549908	0	0	38.07614397	20.87648147	
8		2	Yes	Inner	0	34.68837875	0	0	89.38812819	0	
9		3	Yes	Inner	0	0	0	0	49.99871473	27.58813905	
10		4	Yes	Inner	0	8.895905473	0	0	22.54191114	0	
11		5	Yes	Inner	0	17.79181095	0	51.16810232	0	0	
12	DO1	1	No	Inner	0	0	0	0	3344.823399	803.8615862	
13		2	No	Inner	0	0	0	0	801.6671508	825.504714	
14		3	No	Inner	0	0	0	0	0	928.3573877	
15		4	No	Inner	0	0	0	0	0	250.0795945	
16		5	No	Inner	0	0	0	0	0	3943.030976	
17	DO2	1	No	Inner	0	0	0	0	410.4122884	42.20930013	
18		2	No	Inner	0	0	0	0	11.52533634	2.651510778	41.6386
19		3	No	Inner	0	0	0	0	38.20208402	21.42392402	
20		4	No	Inner	0	0	0	0	60.24855971	71.45131273	
21		5	No	Inner	0	0	0	0	0	426.7695095	
22	KI1	1	Yes	Inner	26.94957737	0	0	0	1361.965315	162.9379989	
23		2	Yes	Inner	40.80056086	0	0	0	226.5029675	20.78537612	
24		3	Yes	Inner	58.94293881	0	0	0	208.8141772	35.72565636	
25		4	Yes	Inner	26.94957737	0	0	0	188.4801602	232.2596791	
26		5	Yes	Inner	183.0260262	0	0	0	446.015026	0	
27	KI2	1	Yes	Inner	0	71.1290243	0	41.08601422	22.28788213	69.32168018	
28		2	Yes	Inner	0	5.61549908	0	0	127.610225	124.7121333	
29		3	Yes	Inner	0	26.62324126	0	0	109.7320328	4.557385076	
30		4	Yes	Inner	0	5.61549908	0	0	100.3901652	0	
31		5	Yes	Inner	0	5.61549908	0	0	29.53955032	0	
32	KO1	1	No	Inner	0	0	0	0	246.7601492	56.47521097	
33		2	No	Inner	0	0	0	0	29.53955032	17.85092737	
34		3	No	Inner	26.94957737	0	0	259.8023409	309.1474729	91.17613625	
35		4	No	Inner	166.4284061	0	0	127.7606938	417.0557612	153.2934902	
36		5	No	Inner	35.81142032	0	0	0	157.0441591	274.2833456	
37	KO2	1	No	Inner	0	86.50376278	0	0	1416.244105	0	
38		2	No	Inner	0	0	0	0	321.4499577	0	

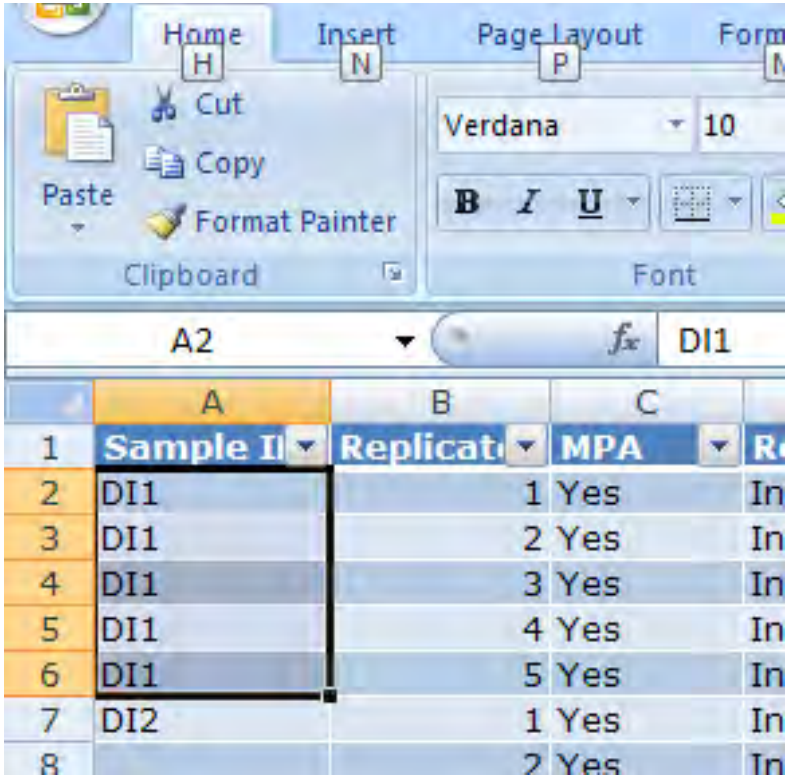
PNP fish pivot chart | PNP fish pivot | PNP Fish Database | PNP Fish Data by Transect | Sheet2

You should have a new database generated that shows fish abundances by transects now. There is only one problem left, for each site ("SampleID") there is only one label with four blank boxes below. We need to fill in the blank boxes below each site label. Unfortunately, there is no automated, easy process to do this, but Excel has some helpful functions to reduce the time required.

22. **Highlight** cells ***A2:A6***

23. Go to the "***Home***" tab on Excel's main menu

- **a. Click** on the drop down box named "***Fill***".
- **b. Choose** the first option "***Down***". (*Notice Excel fills the boxes with the same site label "DI1")*
- **c.** Confirm below.



24. Do the same for all other sites.

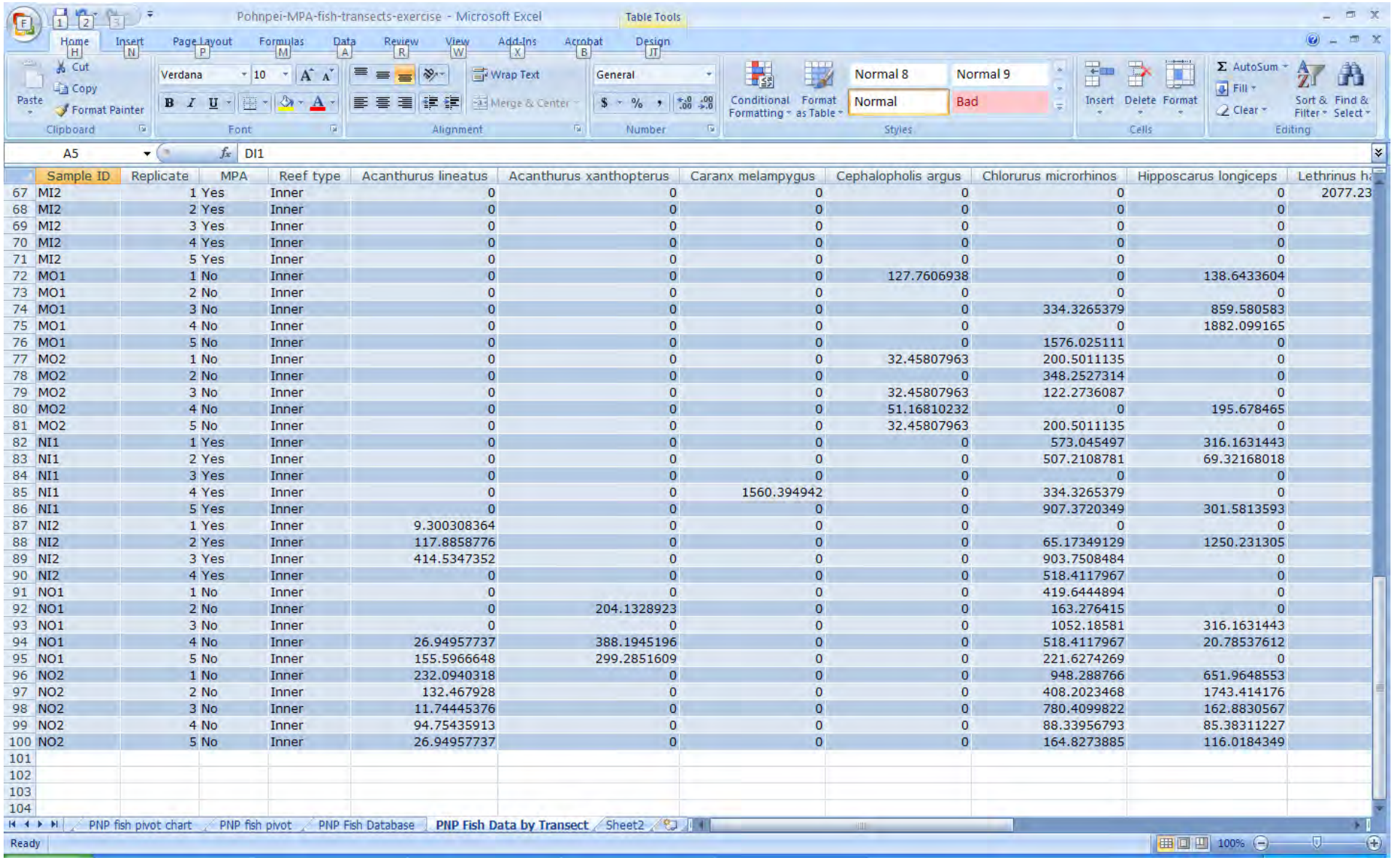
25. Highlight cells ***A7:A11***

- **a. Go to** the "***Fill***" drop down menu and **select** "***down***".
- **b.** Keep on doing this until you fill in all blank boxes on the database.

Tip: Another trick you can use from the keyboard is to click on the first cell with the site name, press Ctrl + C, then move down to the blank cell and press Ctrl + V. This cut and paste works as well.

Ensure that under the heading 'Reef Type' (Column D), only 'Inner' is selected.

When finished confirm you completed data table below.



Pohnpei-MPA-fish-transects-exercise - Microsoft Excel

	Sample ID	Replicate	MPA	Reef type	Acanthurus lineatus	Acanthurus xanthopterus	Caranx melampygus	Cephalopholis argus	Chlorurus microrhinos	Hipposcarus longiceps	Lethrinus ha
67	MI2	1	Yes	Inner	0	0	0	0	0	0	2077.23
68	MI2	2	Yes	Inner	0	0	0	0	0	0	
69	MI2	3	Yes	Inner	0	0	0	0	0	0	
70	MI2	4	Yes	Inner	0	0	0	0	0	0	
71	MI2	5	Yes	Inner	0	0	0	0	0	0	
72	MO1	1	No	Inner	0	0	0	127.7606938	0	138.6433604	
73	MO1	2	No	Inner	0	0	0	0	0	0	
74	MO1	3	No	Inner	0	0	0	0	334.3265379	859.580583	
75	MO1	4	No	Inner	0	0	0	0	0	1882.099165	
76	MO1	5	No	Inner	0	0	0	0	1576.025111	0	
77	MO2	1	No	Inner	0	0	0	32.45807963	200.5011135	0	
78	MO2	2	No	Inner	0	0	0	0	348.2527314	0	
79	MO2	3	No	Inner	0	0	0	32.45807963	122.2736087	0	
80	MO2	4	No	Inner	0	0	0	51.16810232	0	195.678465	
81	MO2	5	No	Inner	0	0	0	32.45807963	200.5011135	0	
82	NI1	1	Yes	Inner	0	0	0	0	573.045497	316.1631443	
83	NI1	2	Yes	Inner	0	0	0	0	507.2108781	69.32168018	
84	NI1	3	Yes	Inner	0	0	0	0	0	0	
85	NI1	4	Yes	Inner	0	0	1560.394942	0	334.3265379	0	
86	NI1	5	Yes	Inner	0	0	0	0	907.3720349	301.5813593	
87	NI2	1	Yes	Inner	9.300308364	0	0	0	0	0	
88	NI2	2	Yes	Inner	117.8858776	0	0	0	65.17349129	1250.231305	
89	NI2	3	Yes	Inner	414.5347352	0	0	0	903.7508484	0	
90	NI2	4	Yes	Inner	0	0	0	0	518.4117967	0	
91	NO1	1	No	Inner	0	0	0	0	419.6444894	0	
92	NO1	2	No	Inner	0	204.1328923	0	0	163.276415	0	
93	NO1	3	No	Inner	0	0	0	0	1052.18581	316.1631443	
94	NO1	4	No	Inner	26.94957737	388.1945196	0	0	518.4117967	20.78537612	
95	NO1	5	No	Inner	155.5966648	299.2851609	0	0	221.6274269	0	
96	NO2	1	No	Inner	232.0940318	0	0	0	948.288766	651.9648553	
97	NO2	2	No	Inner	132.467928	0	0	0	408.2023468	1743.414176	
98	NO2	3	No	Inner	11.74445376	0	0	0	780.4099822	162.8830567	
99	NO2	4	No	Inner	94.75435913	0	0	0	88.33956793	85.38311227	
100	NO2	5	No	Inner	26.94957737	0	0	0	164.8273885	116.0184349	
101											
102											
103											
104											

PNP fish pivot chart | PNP fish pivot | PNP Fish Database | PNP Fish Data by Transect | Sheet2

Ready

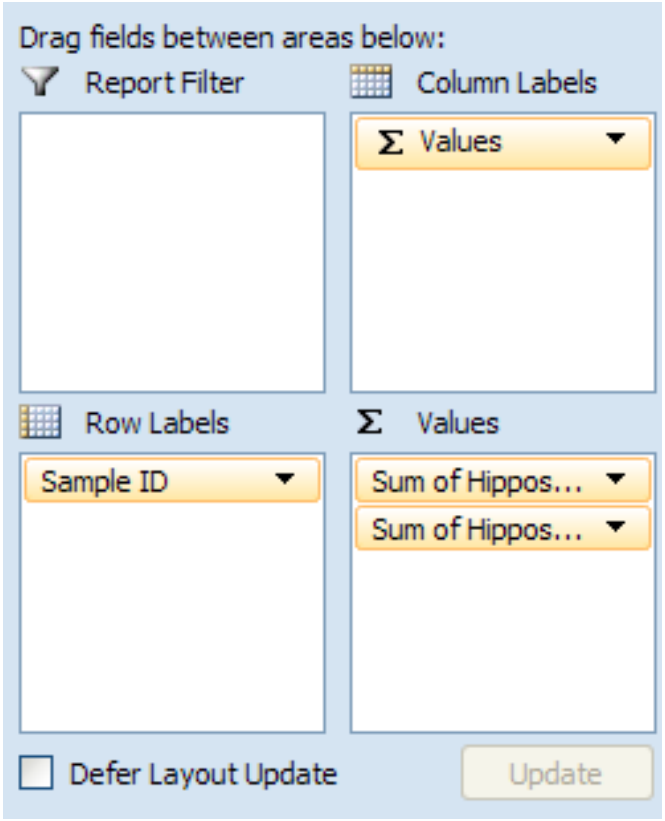
We are now ready to begin examining our statistical confidence.

26. Click anywhere in the table then press ***Ctrl + A*** to highlight all the data.

27. **Insert** a new ***Pivot Table***, and **name** it "***Pivot PNP Fish by Transect***".
    a. **Click** and **drag** the "***Sample ID***" box and **put** it under ***Row Labels***.

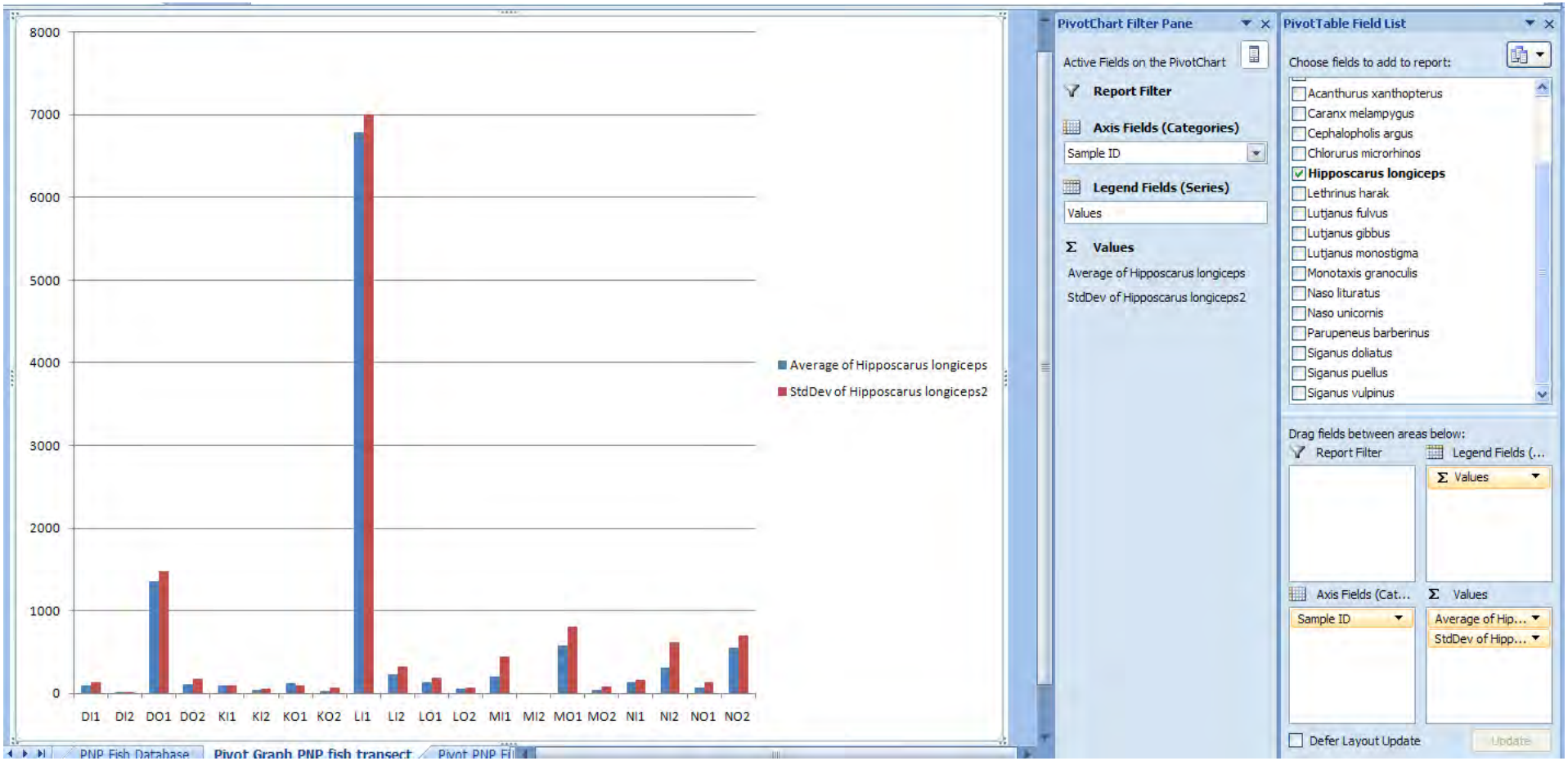
We will first look at one influential fish we found from earlier.

    b. **Click** and **drag** the "***Hipposcarus longiceps***" box and **put** it under ***values***.
    c. **Click** and **drag** the exact same box, and **put** it under ***values***.
    d. Confirm the look of your "***Values***" box below.



28. **Left click** on the top "***Sum of Hipposcarus***" box
    a. **Change** the attributes field to ***Average***.
29. **Left click** on the bottom "***Sum of Hipposcarus***" box
    a. **Change** the attributes field to ***StdDev***.

30. **Click** anywhere inside the main table
31. **Insert** a basic column chart (*the one on the top left of the selection menu*)
    a. **Right click** inside the chart and **move** it into its own spreadsheet
    b. **Rename** the sheet "***Pivot Graph PNP fish transect***".
    c. Confirm.



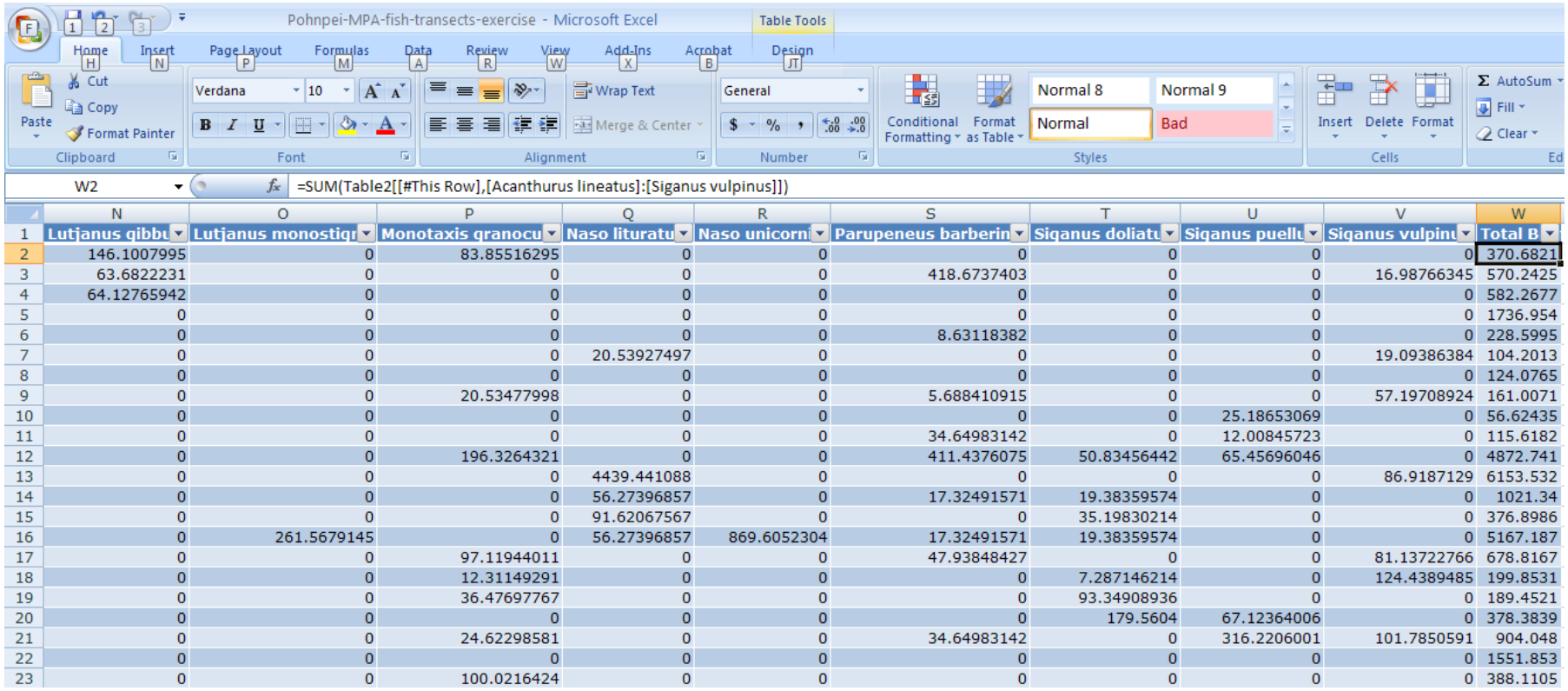
We can clearly see that the standard deviation surrounding these parrotfish estimates for each site is higher than desired, and there is no need to proceed with calculations of statistical power (which we will do in a later exercise).

32. **Repeat** steps ***26b - 30*** and look at "***Naso unicornis***" (*another influential fish we examined before*)

Does the situation differ? Try a few other fish as well. Discuss conclusions.

Clearly at the individual species level there is too much variation in the data to be able to detect significant change over time with statistical confidence. However, we shouldn't worry, fish assemblage data are naturally multivariate in nature. That is, there are many species that make up the total biomass on any given transect, and perhaps we should try to account for all of them simultaneously, rather than individually, one by one. In a later exercise we will analyze the multivariate properties of fish assemblage data. Here, we will attempt a couple last steps to see if we can utilize some properties of the univariate fish dataset.

33. **Return Click** in cell ***V1***
    a. **Name** this cell "***Total Biomass***". (*Notice Excel automatically includes this as part of your data table, and the colors change)*
34. **Click** in cell ***V2***
    a. **Type** the following function "***=sum***(",
    b. **Highlight** all cells in the ***2nd*** row with a fish name on top of them. (*Excel should autofill the entire column once you hit **Enter**)*
35. Confirm.



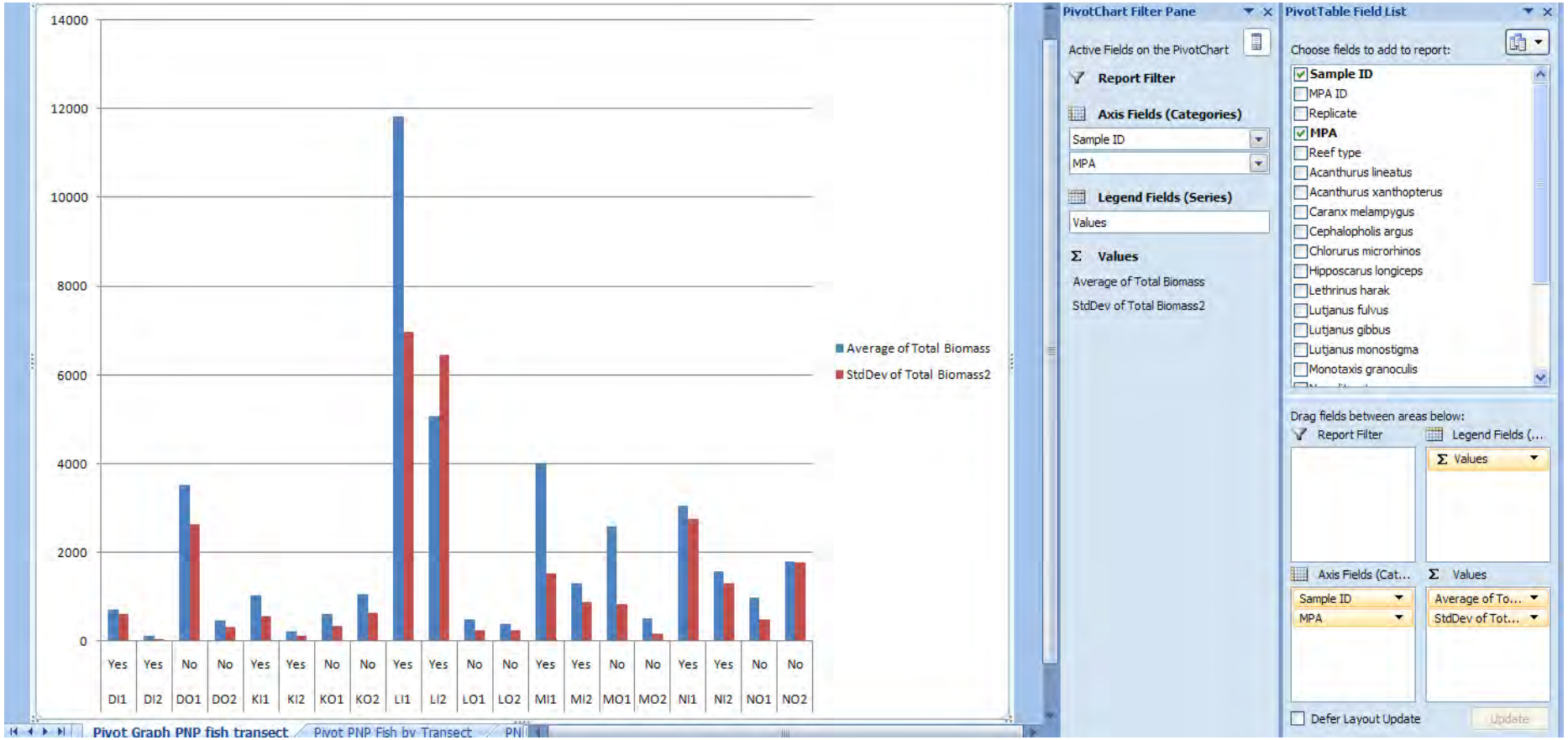
	N	O	P	Q	R	S	T	U	V	W
1	Lutjanus gibbu	Lutjanus monostigu	Monotaxis granocu	Naso lituratu	Naso unicorni	Parupeneus barberin	Siganus doliatu	Siganus puellu	Siganus vulpinu	Total B
2	146.1007995	0	83.85516295	0	0	0	0	0	0	370.6821
3	63.6822231	0	0	0	0	418.6737403	0	0	16.98766345	570.2425
4	64.12765942	0	0	0	0	0	0	0	0	582.2677
5	0	0	0	0	0	0	0	0	0	1736.954
6	0	0	0	0	0	8.63118382	0	0	0	228.5995
7	0	0	0	20.53927497	0	0	0	0	19.09386384	104.2013
8	0	0	0	0	0	0	0	0	0	124.0765
9	0	0	20.53477998	0	0	5.688410915	0	0	57.19708924	161.0071
10	0	0	0	0	0	0	0	25.18653069	0	56.62435
11	0	0	0	0	0	34.64983142	0	12.00845723	0	115.6182
12	0	0	196.3264321	0	0	411.4376075	50.83456442	65.45696046	0	4872.741
13	0	0	0	4439.441088	0	0	0	0	86.9187129	6153.532
14	0	0	0	56.27396857	0	17.32491571	19.38359574	0	0	1021.34
15	0	0	0	91.62067567	0	0	35.19830214	0	0	376.8986
16	0	261.5679145	0	56.27396857	869.6052304	17.32491571	19.38359574	0	0	5167.187
17	0	0	97.11944011	0	0	47.93848427	0	0	81.13722766	678.8167
18	0	0	12.31149291	0	0	0	7.287146214	0	124.4389485	199.8531
19	0	0	36.47697767	0	0	0	93.34908936	0	0	189.4521
20	0	0	0	0	0	0	179.5604	67.12364006	0	378.3839
21	0	0	24.62298581	0	0	34.64983142	0	316.2206001	101.7850591	904.048
22	0	0	0	0	0	0	0	0	0	1551.853
23	0	0	100.0216424	0	0	0	0	0	0	388.1105

36. **Go back** to the "***Pivot Graph PNP fish transect***" worksheet with our graph.

37. **Click** anywhere in the chart to activate the Pivot Chart functions.
38. **Click** on the "***Analyze***" tab in Excel's main menu, then **click** the "***Refresh***" button.

Notice in your "PivotTable Field List" that "Total Fish Biomass" has been added.

39. **Remove** all active boxes from the "***Values***" field by **unchecking** the green marks next to any active fish name you were previously investigating.
40. **Click** and **drag** the "***MPA***" box and place it under "***SampleID***".
41. **Click** and **drag** the "***Total Fish Biomass***" box and place it under "***Values***".
    a. Do this twice so you have two boxes.
    b. **Change** the attributes of each to "***Average***" and "***StdDev***".



We can clearly see that we have improved our confidence interval surrounding our data by utilizing the new field "sum of fish biomass". In many instances the standard deviation appears to be less then 50% of the mean, and appropriate for the calculation of statistical power. However, this trend is not universal, and our conclusion would be to also examine the multivariate properties of these datasets. Both statistical power and multivariate data analyses are approached in a later exercise.
In just a short period of time we have successfully identified island-wide trends associated with Pohnpei's MPA network. We subsequently identified which MPA's seem to be most successful. Finally, we re-formatted our data to understand statistical consideration of our dataset. We are armed with a logical framework and flow to create a report, power point lecture, grant application, or other type of summary that may be necessary.

42. Save your file for future reference, then you can close it.

End of Exercise 4.

Section 2 - Univariate Statistics and graphing the results

Exercise 5 – Simple calculations of statistical power for influential, dependent variables.

Statistical power is defined as a probability (0 to 100%) that data we collect will be able to detect a desired level of change in the abundance or density of coral, fish, or invertebrates in question. If we take just a few measurements our standard deviation will be high and our power will be low. However, when do we know enough is enough so we can balance our logistical and financial constraints with our data needs? Obviously 0% power is not desirable, but 100% is equally unattainable unless sampling effort is increased beyond realistic levels. Studies agree that power should be 70% or higher for detecting a relative 20 – 30 % change in the resource abundance in question (coral, fish, sea cucumbers, etc.). Here we will conduct some very basic power calculations using the free software R (http://www.r-project.org). Of course the topic of statistical power is well developed in the scientific literature, and references are easily attainable from the "Google Scholar" search engine. Here we will touch upon the subject for our needs of assessing data confidence.

You should have already installed the software package "R" on your computer, if you have not do so now. R is a computer language, and interface program, that allows any user to create their own "code" or instructions for data analysis and user interface. A great book to describe R, and provide you with plenty of examples is "The R Book, MJ Crawley (2007). John Wiley & Sons Inc.". Here, we will only use one simple feature of R to generate statistical power estimates. You can navigate to (http://sekhon.berkeley.edu/stats/html/power.t.test.html) *to understand the code (or package) that we will use.*

We will again use Excel as a basis for our inquiries.

Open the file "***Kosrae-benthic-data-example***".

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Sample_ID	Replicate	SurveyDate	CA	CCA	DC	DCA	DCO	DI	FS	HA	HC	OT	R	RB	RC	RCK	SC	SD	SP
2	FMKSA04111	1	9/21/2005	25	0	0	0	0	0	0	5	62.5	0	0	5	0	0	0	0	0
3	FMKSA04111	2	9/21/2005	27.5	0	0	0	0	0	2.5	0	70	0	0	0	0	0	0	0	0
4	FMKSA04111	3	9/21/2005	12.5	0	0	0	0	0	5	0	77.5	0	0	0	0	0	0	0	0
5	FMKSA04111	4	9/21/2005	17.5	0	0	0	0	0	5	5	45	0	0	0	0	0	0	0	0
6	FMKSA04113	1	9/22/2006	12.5	0	0	0	0	0	0	5	72.5	0	0	2.5	0	0	0	0	0
7	FMKSA04113	2	9/22/2006	12.5	0	0	0	0	0	0	0	87.5	0	0	0	0	0	0	0	0
8	FMKSA04113	3	9/22/2006	5	0	0	0	0	0	2.5	0	92.5	0	0	0	0	0	0	0	0
9	FMKSA04113	4	9/22/2006	15	0	0	0	0	0	17.5	2.5	52.5	0	0	0	0	0	0	0	0
10	FMKSA04115	1	9/28/2007	12.5	0	2.5	0	0	0	0	10	60	0	0	0	10	0	0	0	0
11	FMKSA04115	2	9/28/2007	12.5	0	0	0	0	0	0	7.5	77.5	0	0	2.5	0	0	0	0	0
12	FMKSA04115	3	9/28/2007	20	0	5	0	0	0	0	5	55	0	0	0	2.5	0	2.5	0	0
13	FMKSA04115	4	9/28/2007	12.5	0	0	0	0	0	5	17.5	50	0	0	0	12.5	0	0	0	0
14	FMKSA04120	1	10/1/2008	15	0	0	0	0	0	0	2.5	62.5	0	0	5	7.5	0	0	0	0
15	FMKSA04120	2	10/1/2008	10	0	0	0	0	0	0	5	67.5	0	0	0	7.5	0	0	0	0
16	FMKSA04120	3	10/1/2008	7.5	0	0	0	0	0	0	2.5	80	0	0	0	2.5	0	0	0	0
17	FMKSA04120	4	10/1/2008	10	0	0	0	0	0	0	5	72.5	0	0	0	0	0	0	0	0

You can see a very straightforward datasets with "Sample ID", "Replicate", and "Date" to define each sampling event. The remaining codes indicate benthic categories that Kosrae's monitoring program used to collect data. These benthic data were collected using four, 20m long transect lines and noting the benthic life form at each 0.5m mark on the line. Thus, there is a total of four replicate transects with 40 benthic data points collected along each.

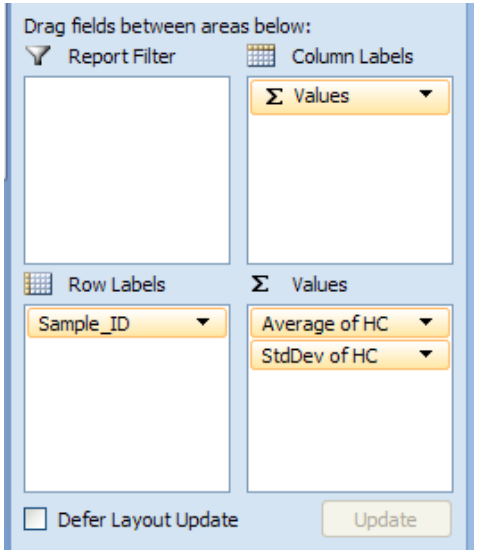
For our purposes we will focus on "Column L" or "HC", which refers to hard coral cover. The numbers below are percent coverages. There are four key elements for calculating and understand statistical power:

1) standard deviations associated with your measurements,
2) required statistical power or confidence,
3) number of replicate samples you have used
4) desired absolute value of change you want to be able to detect.

If you know any of the above 3 values, the simple analyses through "R" will provide you with the calculation of the fourth.

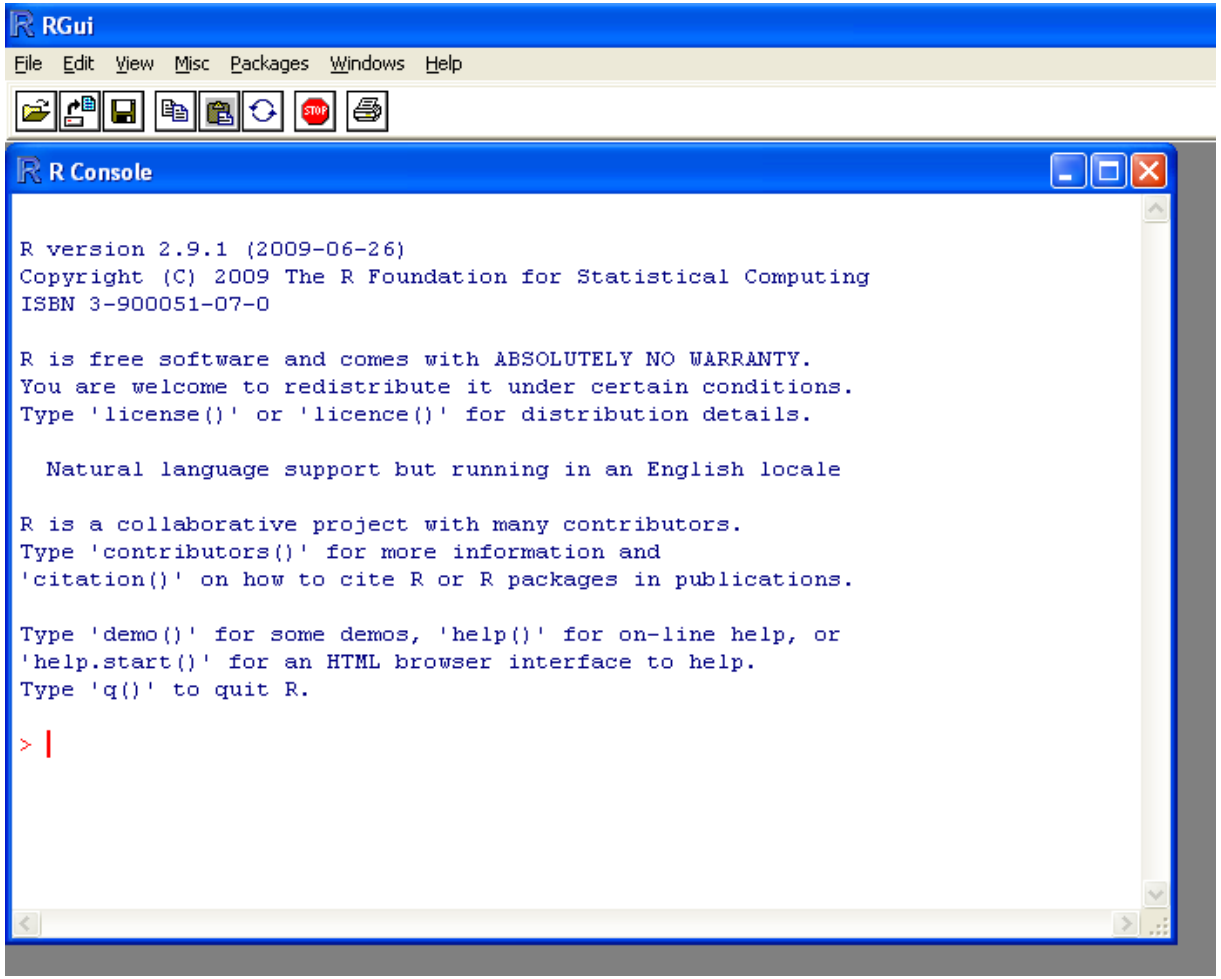
First we will use a Pivot Table to transform the look of our data table for easier interpretation.

1. **Insert** a ***Pivot Table***; call it "***Kosrae-benthic-pivot***".
    a. **Place** "***Sample_ID***" under "***Row labels***", and "***HC***" under "***Values***" two times.
    b. **Change** the attributes of one of the "***HC***" boxes to "***Average***", and the other to "***StDev***".
    c. Confirm below.



Now we have a simple table of each monitoring station with an average and standard deviation of hard coral cover. Get out our scratch paper for now, and note the sample ID and standard deviation for the first row of data, row 5.

2. **Open** the ***R software***.

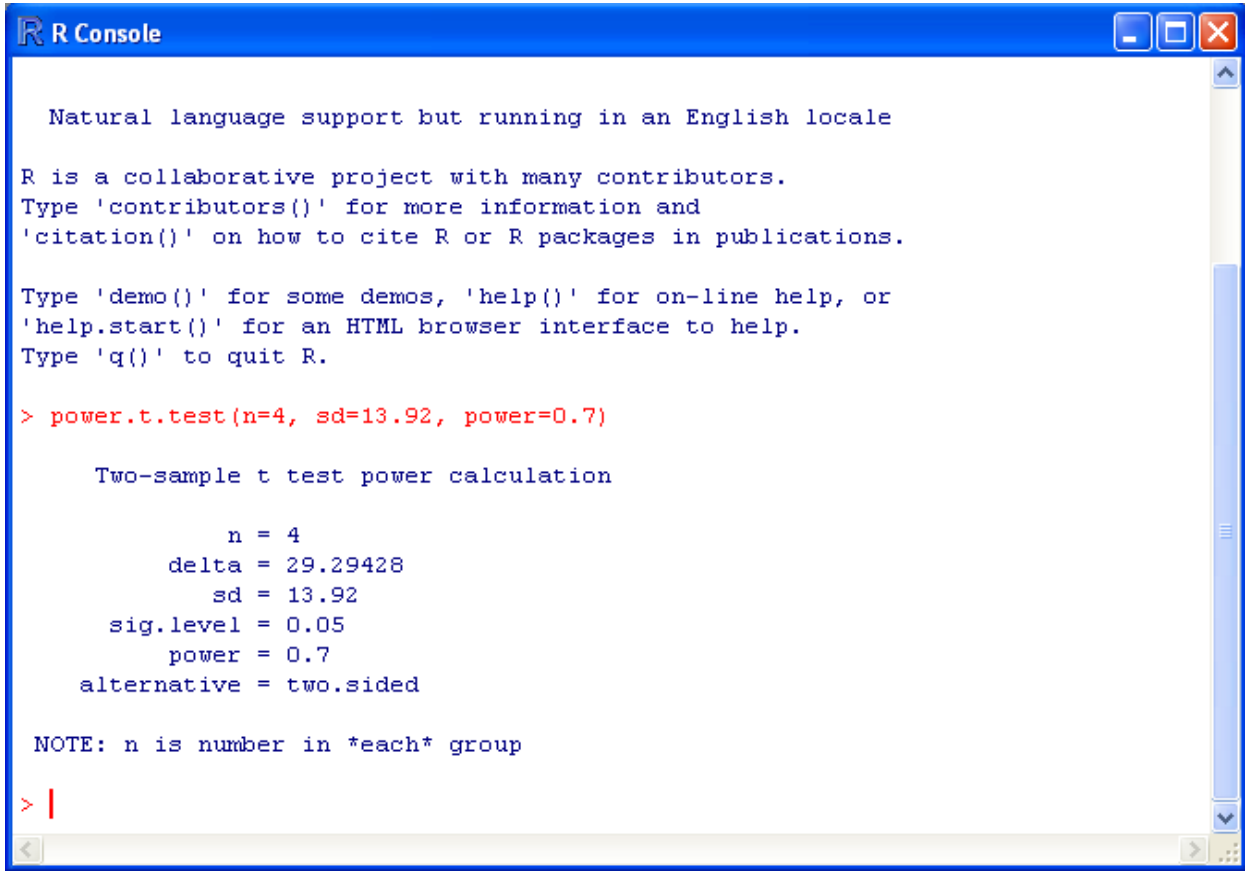


You should have a "**R Console**" dialog box that is ready to accept code to process your queries.
The package for standard statistical power calculations comes pre-loaded in R.

3. **Insert** the ***code*** you learned about from the website. (**power.t.test(n=4, sd=13.92, power=0.7)"**)

We are required to provide 3 of the fours items listed above, remember. So we know our sampling originated from n=4 transects, our sd=13.92 from the excel sheet, and our desired power level (or probability) will be 70% or 0.7.

4. **Press** Enter
5. Confirm with screen shot below.



We can see the results now very clearly. We are interested in the value for "delta" or level of change successfully detected, because we set the values for the rest.
The results suggest that given our sample size and standard deviation we are able to confidently detect a ~30% change in coral cover with statistical significance

6. **Write** the delta value (***29.29***) on your ***scratch*** paper.
7. **Go back** to Excel.

To understanding what our delta value translates into, in terms of percent change, lets put our delta value in perspective with our coral cover value.

   a. **Click** in Cell ***E4*** and **type** the word "***Delta***".

b. **Type** in our value (***29.29***) below in Cell ***E5***.
c. **Click** in Cell ***F4*** and **type** "***Percent Change Detected***".
d. **Click** in Cell **F5** and **type** the following simple math formula "***=(29.29/63.75)*100***".

This takes our "delta" value, divides it by the total coverage of coral, and tells us what percent change we can successfully account for with our sampling design.

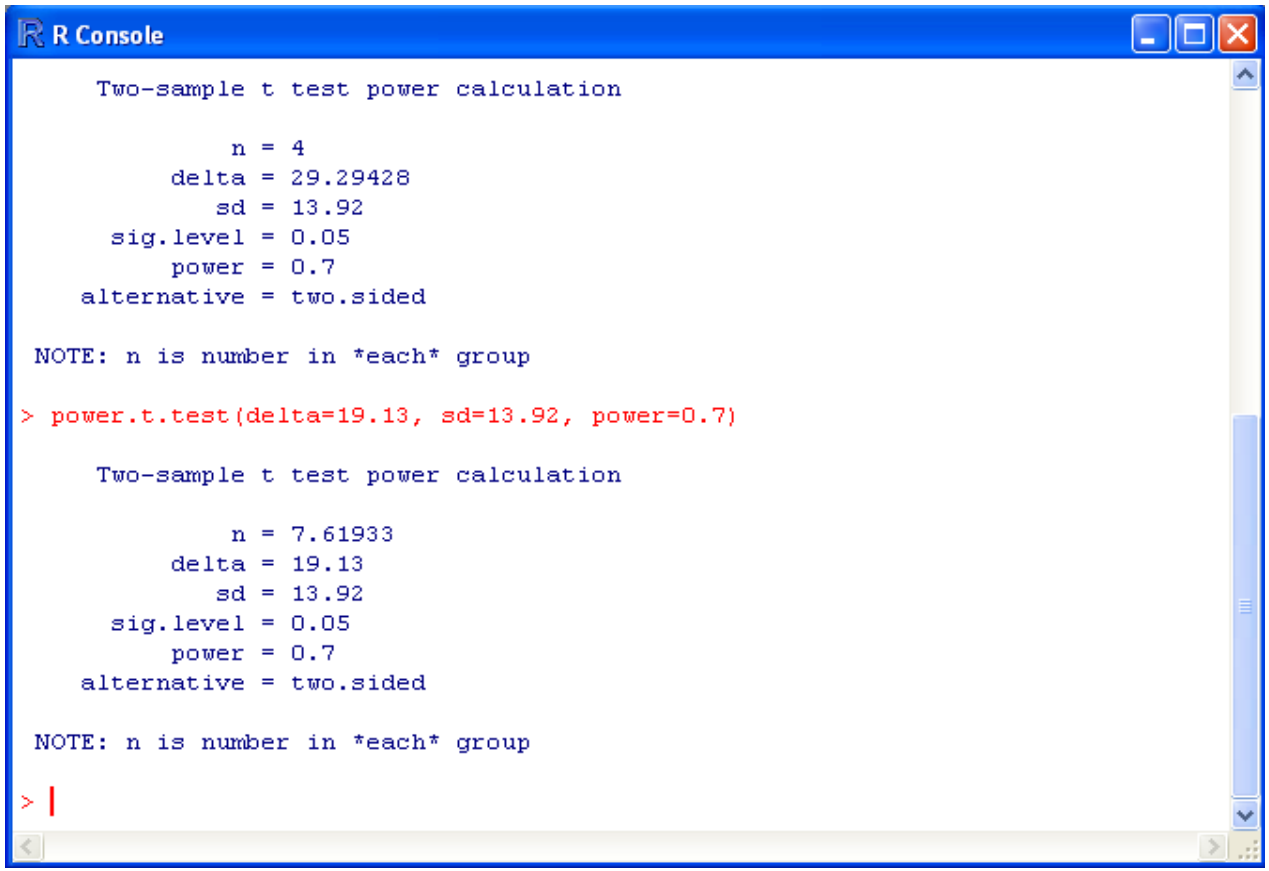
e. Confirm.

	A	B	C	D	E	F	G
1							
2							
3		Data					
4	Sample_ID	Average of HC	StdDev of HC		Delta	Percent Change Detected	
5	FMKSA04111	63.75	13.91941091		29.29	45.94509804	
6	FMKSA04113	76.25	17.96988221				
7	FMKSA04115	60.625	11.96783885				
8	FMKSA04120	70.625	7.465197028				
9	fmksa08110	62.5	7.359800722				
10	FMKSA081101	67.5	9.574271078				
11	FMKSA08112	61.25	8.539125638				
12	FMKSA081121	63.75	5.204164999				
13	FMKSA08116	70	9.789450104				
14	FMKSA0814	56.875	12.47914928				
15	FMKSA0816	71.875	18.41364983				
16	FMKSA08161	76.875	11.43368561				
17	FMKSA0818	65.625	17.60385848				
18	FMKSA08181	63.125	16.37770334				
19	FMKSA0819	63.75	10.50793351				
20	FMKSA13113	36.25	7.772815878				
21	FMKSA13115	36.875	6.884463184				

Notice that only a ~46% change in coral cover can be detected from this first site with statistical confidence, however we desired to detect 30% change in coral cover.
How many replicate samples would we need to do that? Its easy to calculate.
First, recall that the average coral cover for the "FMKSA04111" site is 63.75%, and 30% of that is easily calculated as "19.13".

So, our desired "delta" value is 19.13 and now we want to find out what number of transects we need to reach our goal.

8. **Go back** to the R software.
    a. **Type** in the following code → "***power.t.test(delta=19.13, sd=13.92, power=0.7)***"
    b. **Press** Enter.
    c. Confirm.



Now, let's focus on the value for "n" that was calculated for us (n=7.62). This means that to accomplish our goals we'd need to sample ~8 transects, or basically double the amount of work Kosrae had done.
But, let's think bigger picture. We can see that several surveys were already completed, and perhaps we'd like to know, on average, how did the surveys do at accomplishing their statistical confidence goals.

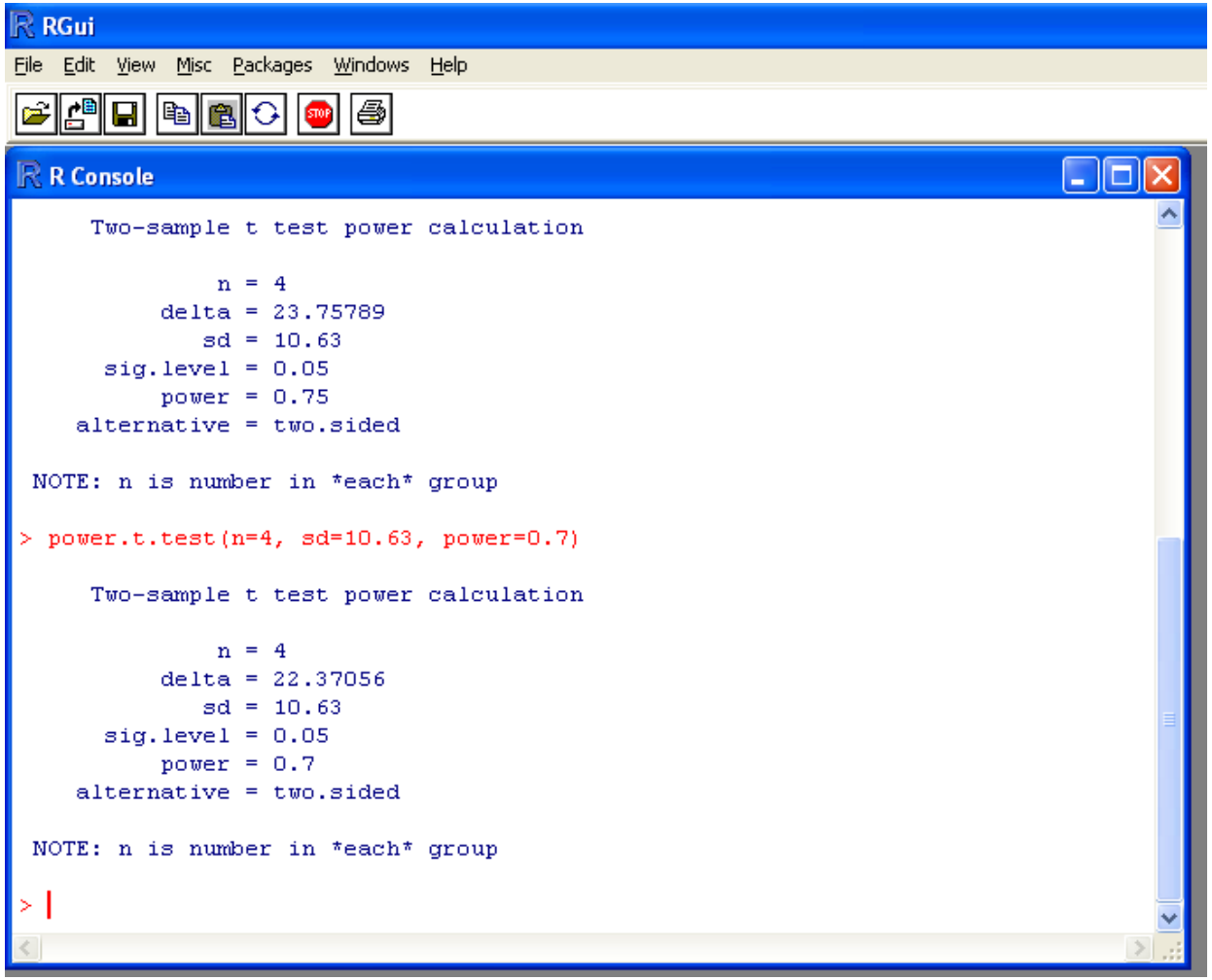
9. **Go back** to Excel.
    a. **Delete** cells ***E5***-***E6*** and ***F5***-***F6*** for now, because we want to look at all sites combined.
    b. In Cell ***E5***, type "***Overall HC Average***"
    c. In ***F5*** type "***Overall HC standard deviation***"
    d. In cell ***E6*** type "***=average(B5:B28)***" (*this takes the overall average of HC*)
    e. In cell ***F6*** type "=average(C5:C28)" (*this takes the overall mean deviation of HC*)
    f. Confirm.

	A	B	C	D	E	F
1						
2						
3		Data				
4	Sample_ID	Average of HC	StdDev of HC			
5	FMKSA04111	63.75	13.91941091		Overall HC average	Overall HC standar deviation
6	FMKSA04113	76.25	17.96988221		60.52083333	10.62544548
7	FMKSA04115	60.625	11.96783885			
8	FMKSA04120	70.625	7.465197028			
9	fmksa08110	62.5	7.359800722			
10	FMKSA081101	67.5	9.574271078			
11	FMKSA08112	61.25	8.539125638			
12	FMKSA081121	63.75	5.204164999			
13	FMKSA08116	70	9.789450104			
14	FMKSA0814	56.875	12.47914928			
15	FMKSA0816	71.875	18.41364983			
16	FMKSA08161	76.875	11.43368561			
17	FMKSA0818	65.625	17.60385848			
18	FMKSA08181	63.125	16.37770334			
19	FMKSA0819	63.75	10.50793351			
20	FMKSA13113	36.25	7.772815878			
21	FMKSA13115	36.875	6.884463184			
22	FMKSA1312	46.875	9.655525189			

Now, note the overall standard deviation on your scratch paper

10. Return to the ***R Software***.
    a. **Type** "***power.t.test(n=4, sd=10.63, power=0.7)***"

b. Confirm.



We can see that based upon all of the sites Kosrae's team surveyed, a ~22% change is confidently detected in HC.
To understanding what our delta value translates into, in terms of percent change, let's put our delta value in perspective with our coral cover value.

11. Go back to Excel.

- **a. Click** in Cell ***G4*** and **write** the word "***Delta***".
- **b. Type** in our value (***22.37***) below in Cell ***G5***.
- **c. Click** in Cell ***H4*** and **write** "***Percent Change Detected***".

d. Click in Cell ***H5*** and **write** the following simple math formula "***=(22.37/60.52)*100***".

This takes our "delta" value, divides it by the mean coverage of coral, and tells us what percent change that was detected, on average, with our sampling design.

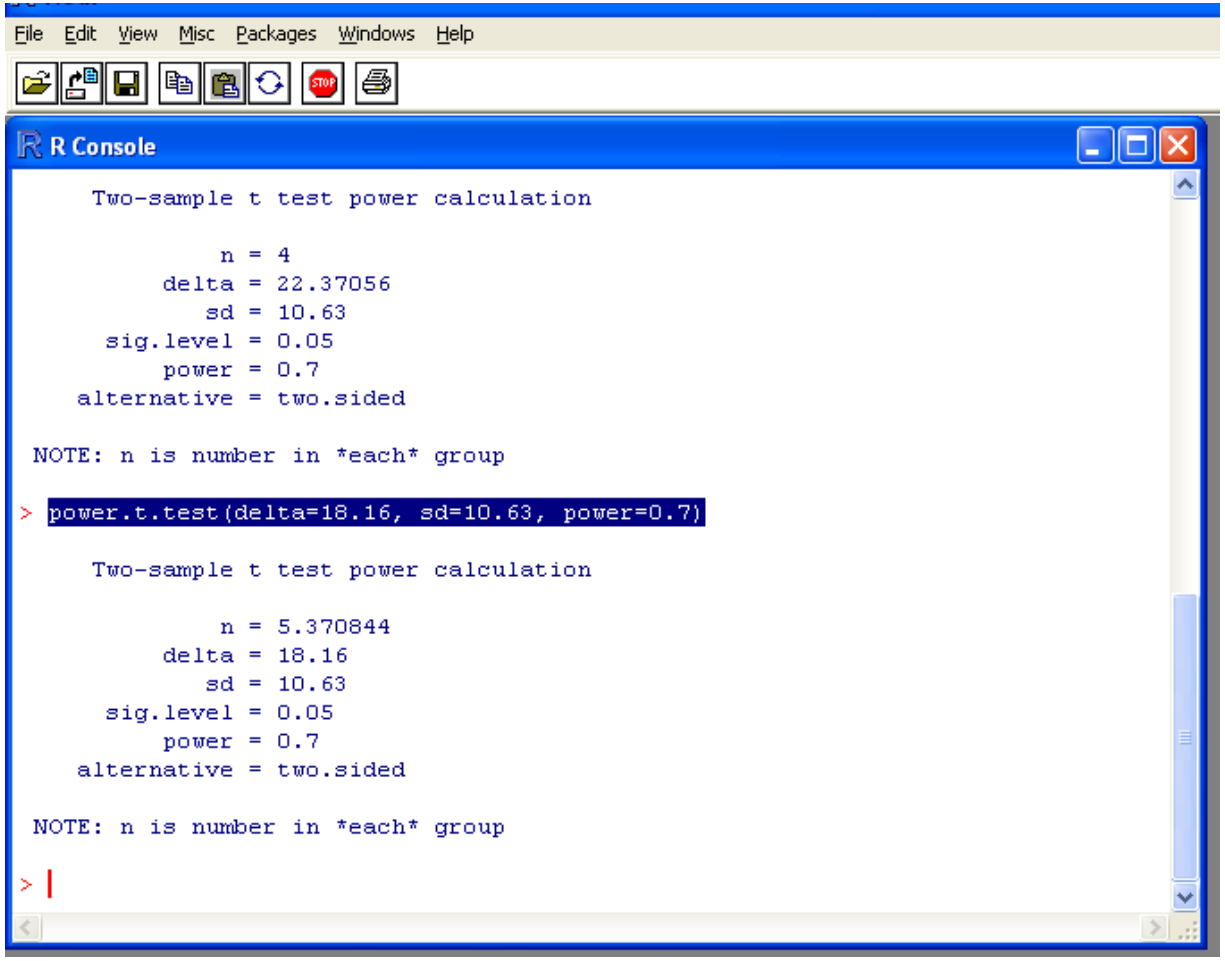
e. Confirm.

	A	B	C	D	E	F	G	H	I
1									
2									
3		Data							
4	Sample_ID	Average of HC	StdDev of HC						
5	FMKSA04111	63.75	13.91941091		Overall HC average	Overall HC standar deviation	Delta	Percent Change Detected	
6	FMKSA04113	76.25	17.96988221		60.52083333	10.62544548	22.37	36.96299	
7	FMKSA04115	60.625	11.96783885						
8	FMKSA04120	70.625	7.465197028						
9	fmksa08110	62.5	7.359800722						
10	FMKSA081101	67.5	9.574271078						
11	FMKSA08112	61.25	8.539125638						
12	FMKSA081121	63.75	5.204164999						
13	FMKSA08116	70	9.789450104						
14	FMKSA0814	56.875	12.47914928						
15	FMKSA0816	71.875	18.41364983						
16	FMKSA08161	76.875	11.43368561						
17	FMKSA0818	65.625	17.60385848						
18	FMKSA08181	63.125	16.37770334						
19	FMKSA0819	63.75	10.50793351						
20	FMKSA13113	36.25	7.772815878						
21	FMKSA13115	36.875	6.884463184						
22	FMKSA1312	46.875	9.655525189						
23	FMKSA1319	36.25	13.1497782						
24	fmksa16110	52.5	5.400617249						
25	FMKSA161101	59.375	12.14066857						
26	FMKSA16112	60.625	3.145764348						
27	FMKSA161121	61.25	7.772815878						
28	FMKSA16116	68.125	10.48312135						
29	Grand Total	60.52083333	14.99524779						

We can see that Kosrae is successfully able to detect a ~37% change in HC, should one occur, with confidence using their sampling design.

Recall that our goals were to detect a 30% change in HC.

12. **Go back** to ***R*** and Calculate how many transects would be required to improve our confidence just a bit to attain these goals.
    a. **Set** our ***delta value*** to ***30%*** of the average estimate of coral cover, (*or 30% of 60.52, or 18.16*)
    b. **Type** the following: "***power.t.test(delta=18.16, sd=10.63, power=0.7)***"
    c. Confirm.



You can see that with just a bit more effort (~5 transect) Kosrae could successfully meet the goals we laid out.
We are finished with the current exercise. Note that we can easily substitute fish counts, abundances, biomass, algae coverage, or whatever our key ecological metric is within any survey. This exercise was intended to provide you an example to follow for making future calculations on your own.

Also keep in mind that many ecological datasets are multivariate in nature, and statistical power, by definition, only accounts for one variable. Typically, monitoring programs select one key variable, such as coral coverage or other abundant benthic organisms, to examine. The results will indicate whether or not your level of replication is sufficient, generally. This is usually a good start prior to moving into multivariate considerations of the datasets, presented below.

End of Exercise 5

Exercise 6.1 – An introduction to creating report-quality graphs and preparing data for univariate statistical analyses

So far we have been using Excel to generate our visual graphs because of the easy manipulation of data through the PivotTable and PivotChart functions. However, once we have completed our initial investigations and have decided upon the influential trends and what graphs best show them, we often desire to create professional, publication-quality graphs for our grant applications and reports. In this exercise the Sigma Plot software is introduced. This software platform is one easy approach that many research scientists use to generate professional figures and conduct basic accompanying statistical analyses. We will make a series of graphs that correspond to investigation of coral reef monitoring trends that have emerged in the Commonwealth of the Northern Mariana Islands (CNMI).

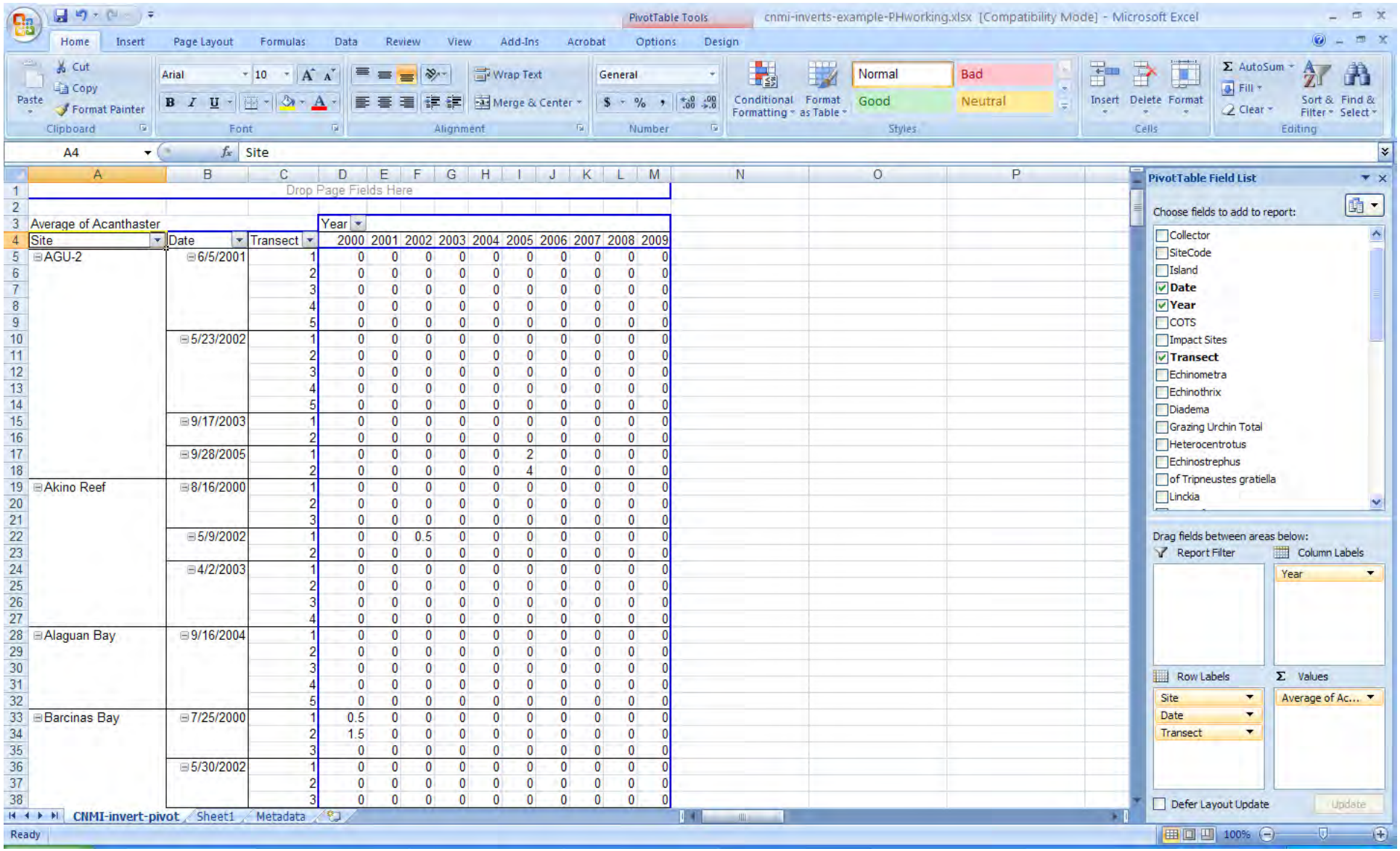
1. **Open** Excel
   a. **Open** the file "***cnmi-inverts-example***".

These are macroinvertebrate count data that were collected along 50m x 4m belt transects over the past 9 years. Each row corresponds to one individual transect. Look at the database, and the corresponding metadata sheet to understand how these data are arranged. Note that columns G and H will be explained later in this exercise, they pertain to our preliminary findings that we will go through.

CNMI's program has experienced several years of higher than average Acanthaster planci abundances, and associated coral damage. We will use the collected data to understand what has happened and what potential consequences and management actions are.

2. **Make** a ***PivotTable***.
   a. **Rename** the new worksheet "***CNMI-invert-pivot***".
3. **Drag** "***Year***" under "***Column Labels***"
   a. **Scroll down** to "**Acanthaster**" and **drag** it under "***Values***".
   b. **Left click** on it and **change** the "***Value field setting***" to "***Average***".
4. **Drag** "***Site***", "***Date***", and "***Transect***" under the ***Row Labels***, in that order.
5. **Right click** anywhere in the ***PivotTable*** and
   a. **Select** "***Pivot Table Options***".
6. On the "***Layout & Format***" tab **put a check** next to the box "***For empty cells show***:"
   a. **Put** a "***0***" (*zero*) in the space.

7. On the "***Totals and Filters***" tab **uncheck** "***Show grand totals***" for columns and rows.
8. On the "***Display***" tab **check** "***Classic PivotTable layout***" option.
9. **Right click** in cell "***A4***" and **go to** "***Field Settings***"
   a. **select** "***None***" under subtotals.
10. **Repeat *Step 9*** for Cells "***B4***" and "***C4***". Confirm.



Our table now has the population density estimates for coral-eating starfish during each year. These are the data and proper format required for Sigma Plot to produce our desired graph.

11. Click on the dropdown menu for "***Year***" in the ***PivotTable*** (*cell D3*)

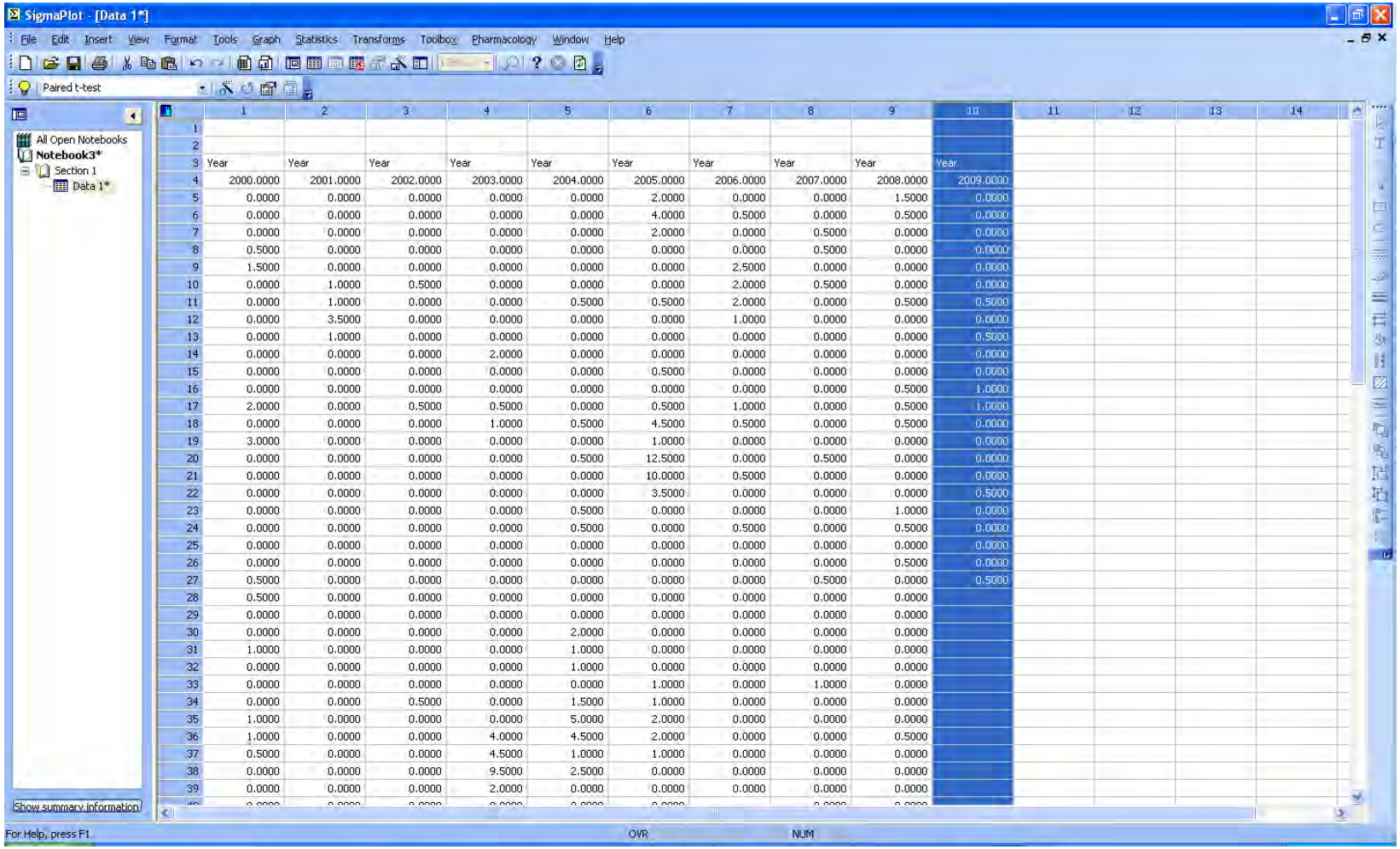
- **a. Transfer** our data year by year. (*Check only the box next to "2000" first*)
- **b.** Confirm.

	A	B	C	D
1				
2				
3	Average of Acanthaster			Year
4	Site	Date	Transect	2000
5	Akino Reef	8/16/2000	1	0
6			2	0
7			3	0
8	Barcinas Bay	7/25/2000	1	0.5
9			2	1.5
10			3	0
11	Barcinus Bay #1	7/26/2000	1	0
12			2	0
13			3	0
14	Bird Island	7/24/2000	1	0
15			2	0
16			3	0
17	Boy Scout	6/28/2000	1	2
18			2	0
19			3	3
20	Coral Gardens	7/26/2000	1	0
21			2	0
22			3	0
23	Coral Ocean Point	11/24/2000	1	0
24			2	0
25			3	0
26	Dynasty	7/27/2000	1	0
27			2	0.5
28			3	0.5
29	Iota North	7/28/2000	1	0
30			2	0
31			3	1
32	Iota South	7/24/2000	1	0
33			2	0
34			3	0
35	Lau #2	1/24/2000	1	1
36			2	1
37			3	0.5
38	Obyan	9/23/2000	1	0

CNMI-invert-pivot | Sheet1 | Metadata

We have to transfer our data on a year-by-year basis because Excel has put in “0” for all empty boxes, even if no surveys were conducted. It’s easy to do.

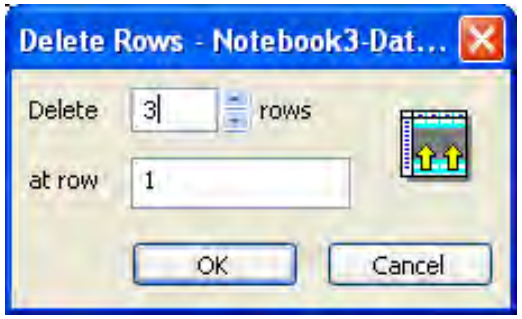
12. **Right click** on “***Column D***”
    a. **Select** “***Copy***”.
13. **Open *Sigma Plot***
    a. **Start** a new notebook.
14. **Right click** on “***Column 1***”
    a. **Choose** paste.
15. Do this for all years, then confirm.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2														
3	Year	Year	Year	Year	Year	Year	Year	Year	Year	Year				
4	2000.0000	2001.0000	2002.0000	2003.0000	2004.0000	2005.0000	2006.0000	2007.0000	2008.0000	2009.0000				
5	0.0000	0.0000	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	1.5000	0.0000				
6	0.0000	0.0000	0.0000	0.0000	0.0000	4.0000	0.5000	0.0000	0.5000	0.0000				
7	0.0000	0.0000	0.0000	0.0000	0.0000	2.0000	0.0000	0.5000	0.0000	0.0000				
8	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.0000				
9	1.5000	0.0000	0.0000	0.0000	0.0000	0.0000	2.5000	0.0000	0.0000	0.0000				
10	0.0000	1.0000	0.5000	0.0000	0.0000	0.0000	2.0000	0.5000	0.0000	0.0000				
11	0.0000	1.0000	0.0000	0.0000	0.5000	0.5000	2.0000	0.0000	0.5000	0.5000				
12	0.0000	3.5000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000				
13	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000				
14	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
15	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.0000	0.0000	0.0000				
16	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	1.0000				
17	2.0000	0.0000	0.5000	0.5000	0.0000	0.5000	1.0000	0.0000	0.5000	1.0000				
18	0.0000	0.0000	0.0000	1.0000	0.5000	4.5000	0.5000	0.0000	0.5000	0.0000				
19	3.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000				
20	0.0000	0.0000	0.0000	0.0000	0.5000	12.5000	0.0000	0.5000	0.0000	0.0000				
21	0.0000	0.0000	0.0000	0.0000	0.0000	10.0000	0.5000	0.0000	0.0000	0.0000				
22	0.0000	0.0000	0.0000	0.0000	0.0000	3.5000	0.0000	0.0000	0.0000	0.5000				
23	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.0000	0.0000	1.0000	0.0000				
24	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.5000	0.0000	0.5000	0.0000				
25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
26	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000				
27	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.5000				
28	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000					
29	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000					
30	0.0000	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	0.0000	0.0000					
31	1.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000					
32	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000					
33	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	1.0000	0.0000					
34	0.0000	0.0000	0.5000	0.0000	1.5000	1.0000	0.0000	0.0000	0.0000					
35	1.0000	0.0000	0.0000	0.0000	5.0000	2.0000	0.0000	0.0000	0.0000					
36	1.0000	0.0000	0.0000	4.0000	4.5000	2.0000	0.0000	0.0000	0.5000					
37	0.5000	0.0000	0.0000	4.5000	1.0000	1.0000	0.0000	0.0000	0.0000					
38	0.0000	0.0000	0.0000	9.5000	2.5000	0.0000	0.0000	0.0000	0.0000					
39	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	0.0000	0.0000	0.0000					

Now we can clean this up a bit before starting our graph and statistical analyses.

16. **Right click** on "***Row 1***"
    a. **Choose** "***Delete Rows***".
    b. **Delete** rows ***1 – 3*** (*so choose to delete "3" rows, starting "at row" 1*)



Finally, let's promote our years to official column titles.

17. **Right click** on ***column 1***
    a. **Choose** "***Column Titles***".

    b. **Click** on the ***promote button*** to move the text heading of the first column up.

Notice on your datasheet that "2000" has been promoted to a column title.

18. **Click** on "***Next***"
    a. **Promote** the names for ***columns 2-10***.
    b. **Close** the dialog box.

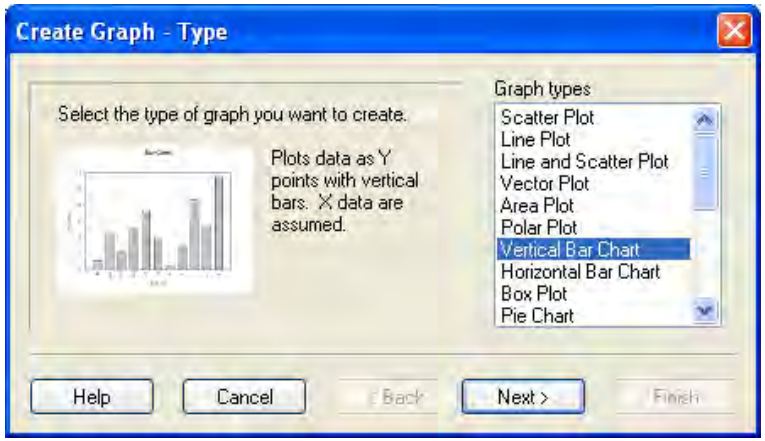
We will now make headers to define our different years of data.

19. **Click** on the ***first*** cell under "***Column 11***".
    a. **Type** the word "***Year***".
20. In the cell under "Year" **type** in "***2000***", then "***2001***" in the cell under that
    a. **Continue** until "***2009***".
21. **Promote** "***Year***" to a column title as we just did before.
22. Confirm.

	1-2000.0000	2-2001.0000	3-2002.0000	4-2003.0000	5-2004.0000	6-2005.0000	7-2006.0000	8-2007.0000	9-2008.0000	10-2009.0000	11-Year
1	0.0000	0.0000	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	1.5000	0.0000	2000.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	4.0000	0.5000	0.0000	0.5000	0.0000	2001.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	2.0000	0.0000	0.5000	0.0000	0.0000	2002.0000
4	0.5000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	0.0000	0.0000	2003.0000
5	1.5000	0.0000	0.0000	0.0000	0.0000	0.0000	2.5000	0.0000	0.0000	0.0000	2004.0000
6	0.0000	1.0000	0.5000	0.0000	0.0000	0.0000	2.0000	0.5000	0.0000	0.0000	2005.0000
7	0.0000	1.0000	0.0000	0.0000	0.5000	0.5000	2.0000	0.0000	0.5000	0.5000	2006.0000
8	0.0000	3.5000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	2007.0000
9	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000	2008.0000
10	0.0000	0.0000	0.0000	2.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2009.0000

Now we are ready to create a simple bar chart.

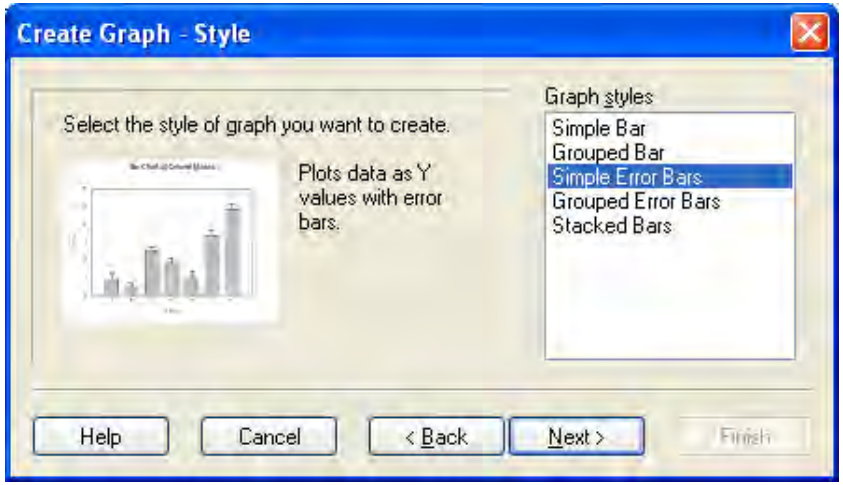
23. **Go to** the "***Graph***" main menu on the top
    a. **Scroll** down to "***Create Graph***".
24. **Choose** "***Vertical Bar Chart***".



a. **Click** next.

For this example we have just a simple bar chart with error bars,

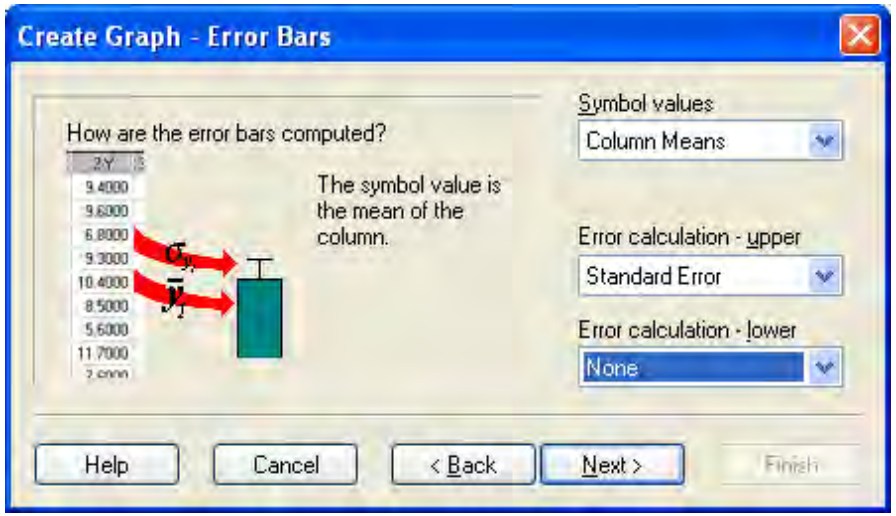
25. **Choose** "***Simple Error Bars***".



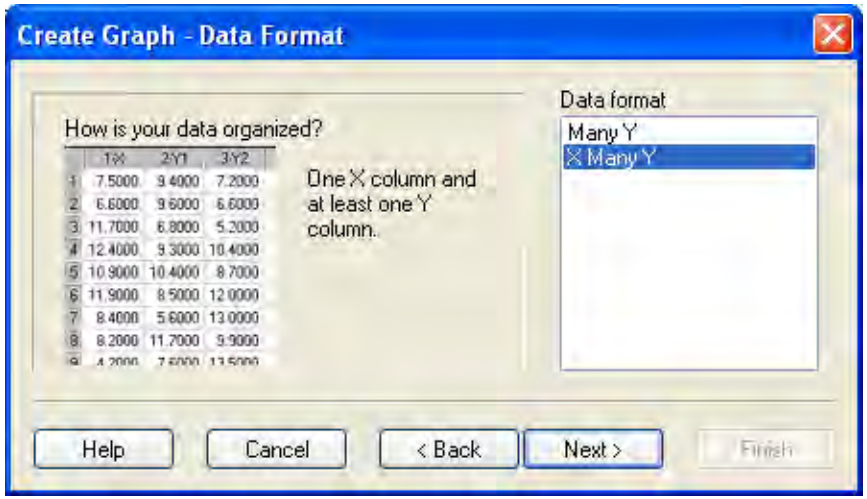
a. Click next.

We want the bars in our chart to represent "Column Means", and let's make errors bars that represent "Standard Error".

26. **Choose** "***None***" for the lower error bars (*these are redundant*)
    a. **Click** next.



27. For ***data format*** **select** "***X Many Y***".

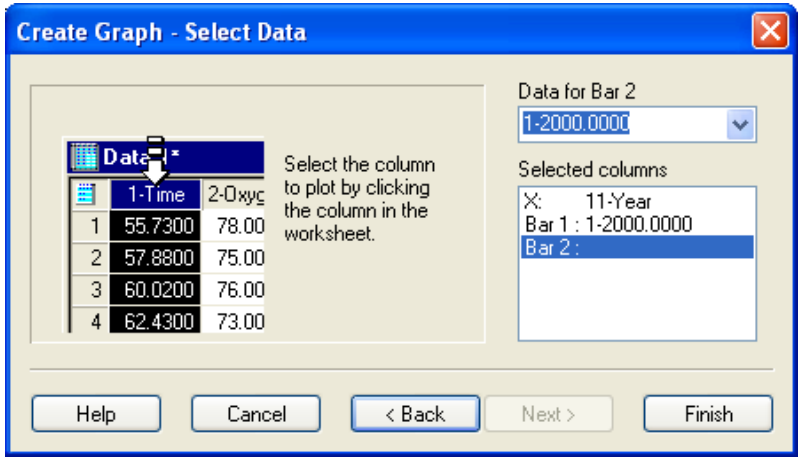


a. Click next.

Now Sigma Plot is asking for the data.

28. In the "***Data for X***" **choose** ***column 11*** ("*Year*")

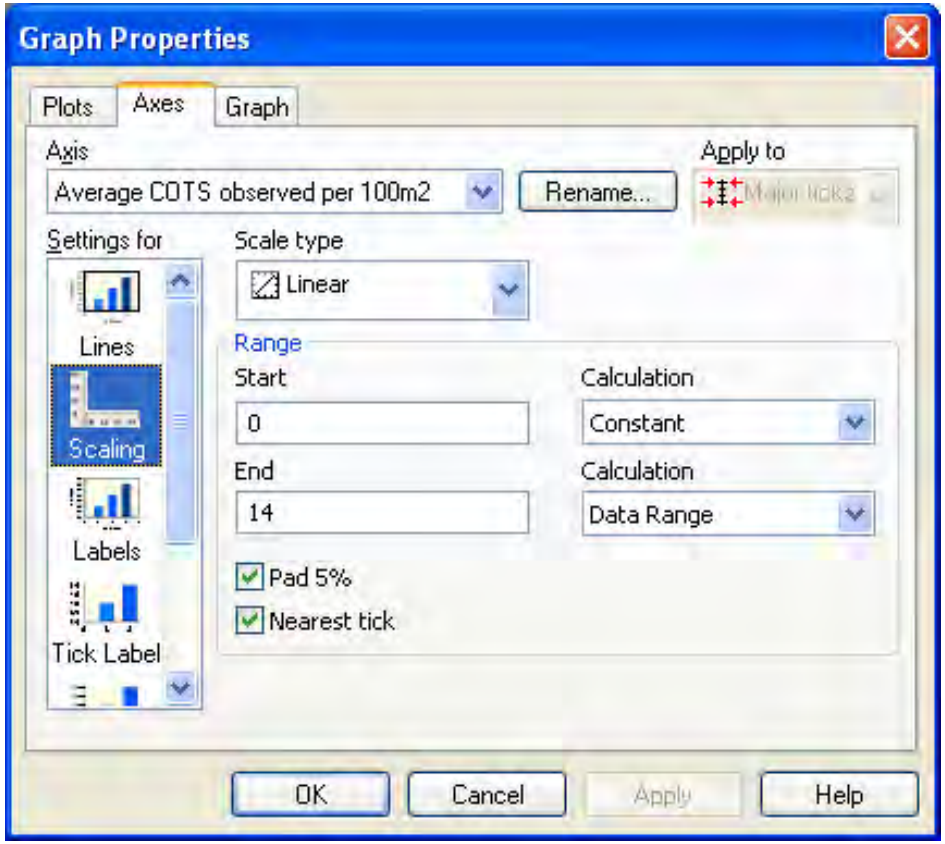
a. "***Bar 1***" **choose** "***2000***".



   b. **Repeat** until you reach "***Bar 10***" and have **highlighted** "***2009***".
   c. **Click** Finish.

The initial look of the graph that is created is relatively unimpressive, but this is easy to change.

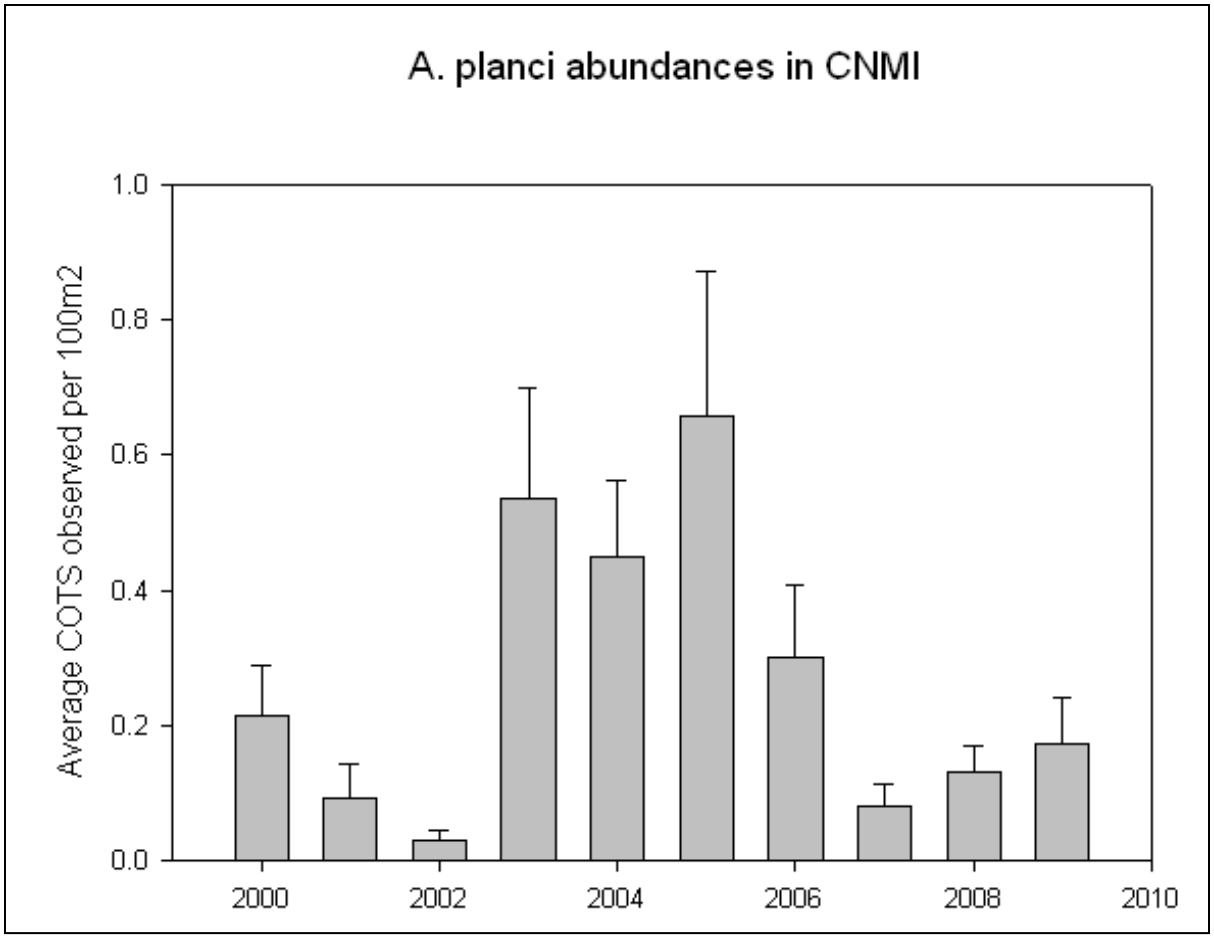
29. In the "***Zoom***" box on top
   a. **Change** the value "***50***%" to "***100***%".
30. **Click** on "***2D Graph 2***"
   a. **Change** the title to "***A. planci abundances in CNMI***"
31. **Click** on "***Y Data***"
   a. **Change** this to "***Average COTS observed per 100m^2***".
32. **Delete** "***X Data***" and the ***legend*** below showing "***Plot 1***".
33. **Double click** on the ***vertical axis numbers***



a. In the "***End***" box **change** the "***14***" to a "***1***".

b. **Click** OK (*take a moment to look at the quality and information presented in just a few easy steps*)

Sigma Plot allows you to export these graphs in raster or vector formats, to preserve high resolution images for your reports or grant applications.



We can summarize that higher than average A. planci abundances were evident in the CNMI between 2003 and 2006. We now wish to understand the ecological consequences of high starfish abundances in terms of our other datasets, and eventually look at recovery.

Save your work. Keep your files open as they are needed for exercise 6.2.

End of Exercise 6.1

Exercise 6.2 – Conducting basic univariate statistical analyses and producing informative, professional quality graphs to show your trends

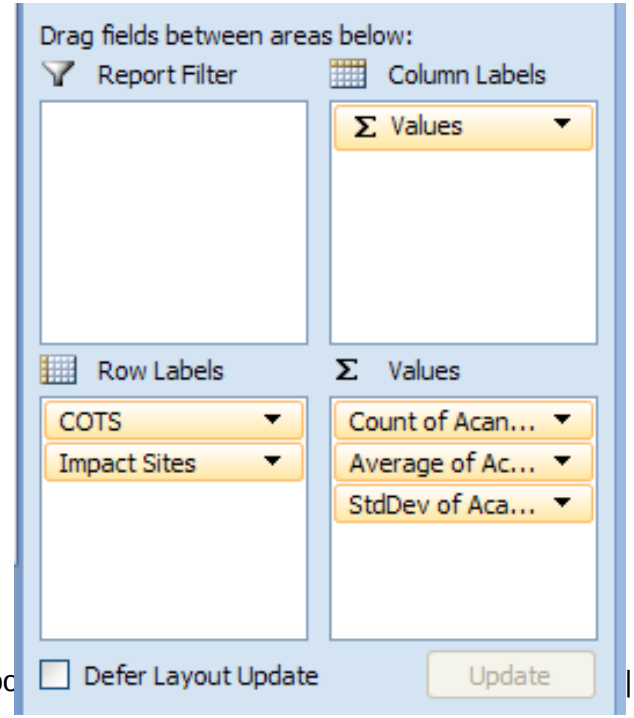
1. **Go back** to our Excel file
   a. **Make** the ***main database*** sheet active.

Notice column G, which is named "COTS". Click on the drop down menu and notice there are three choices: "Before", "During", and "After". These indicate that data were collected before (i.e, from 2000 – 2003), during (2003 – 2006), and after (2006 – 2009) high COTS activity.

Also notice Column H "Impact Sites". The predator starfish were not seen in high abundances at all sites where monitoring was conducted at. "No" indicates that no increase in COTS abundances was evident and "Yes" means high populations were recorded.

We will explore another, more simplified format for transferring data into Sigma Plot for further graphing and analyses of CNMI's database.

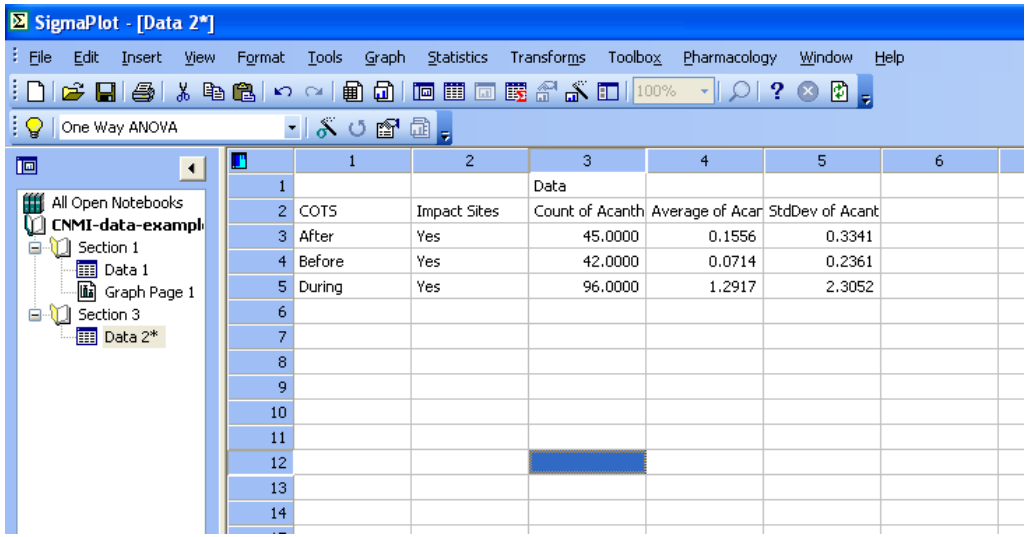
2. **Go back** to our ***PivotTable*** sheet.
3. **Remove** all "Column", "Row", and "Values" from the boxes on the lower right.
4. **Choose** "***COTS***" and "***Impact Sites***" for new row labels in that order.
   a. **Drag** "*Acanthaster*" under "***Values***" ***3 times***.
5. **Left click** the first "Acanthaster"
   a. **Select** "***Field Attributes***" and then **select** "***Count***".
6. **Set** the second to "***Average***".
7. **Set** the third to "***StdDev***".
8. **Right click** on Cell "***A4***" (or "COTS") and
   a. **Choose** "***Field Settings***"
   b. **Check** "***None***" under subtotals and filters.
9. **Repeat** previous step for "***Impact Sites***".
10. Confirm



We again have data ready for Sigma Plot, in a simplified, summarized format. Sigma Plot can handle raw data or summarized, a major benefit for us. Take a moment to understand what is on our datasheet. The "Count" function in excel adds up all cases where data was collected, regardless of the value (i.e., regardless of how many COTS we saw on the transect line, Excel gives a value of 1 for every data entry). Thus, the "Count" is our sample size (n), or total number of transects that were surveyed in each category. The average and standard deviation are self explanatory.

Lets filter our data and begin to transfer to Sigma Plot. Let's first consider only the "Impact Sites" where increase COTS abundances were noted.

11. **Click** on the drop down menu next to "***Impact Sites***"
    a. Check only the "Yes" box.
12. **Highlight** all of the cells in our Pivot Table
    a. **Copy** the data.
13. **Open** (*or return*) ***Sigma Plot***.
14. **Right click** on "***Section 1***" in the panel on the left hand side
    a. **Scroll down** to "***New***", and **choose** "***Worksheet***".
15. **Right click** in cell ***1,1*** and **choose** "***Paste***".
16. Confirm.

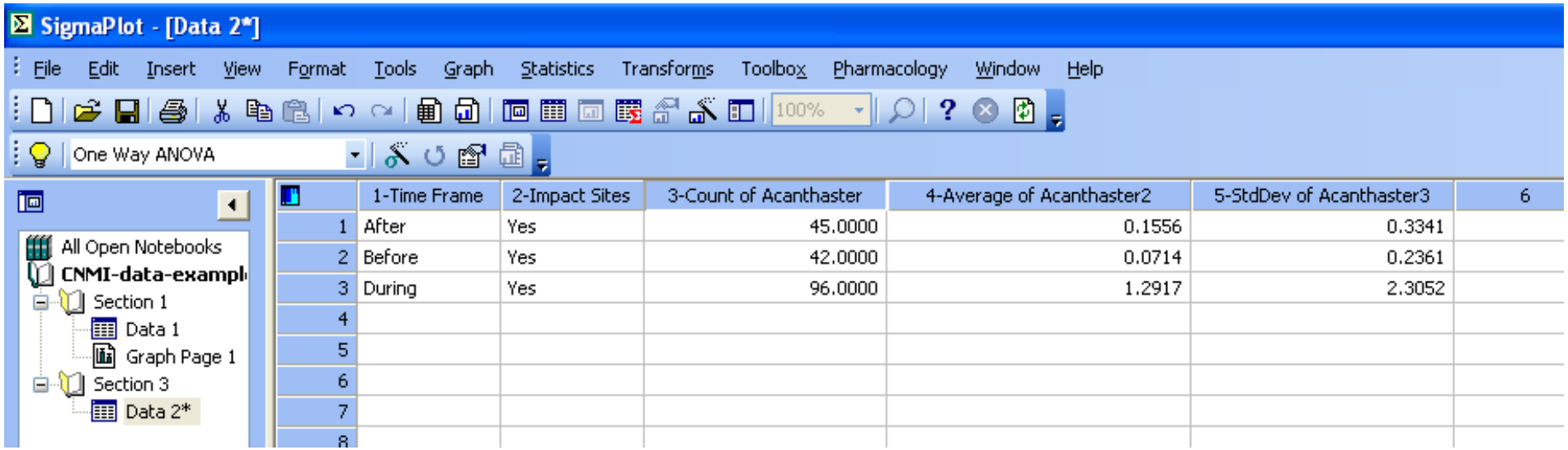


	1	2	3	4	5	6
1			Data			
2	COTS	Impact Sites	Count of Acanth	Average of Acar	StdDev of Acant	
3	After	Yes	45.0000	0.1556	0.3341	
4	Before	Yes	42.0000	0.0714	0.2361	
5	During	Yes	96.0000	1.2917	2.3052	
6						
7						
8						
9						
10						
11						
12						
13						
14						

17. **Right click** on ***row 1***, and **delete** this entire row.
    a. **Rename** cell 1,1 from "***COTS***" to "***Time Frame***".

The values below indicate our time frame of observation.

18. **Right click** on ***Column 1***
    a. **Choose** "***Column Titles***"
19. **Promote** the column headings to titles for all 5 columns.
20. Confirm.

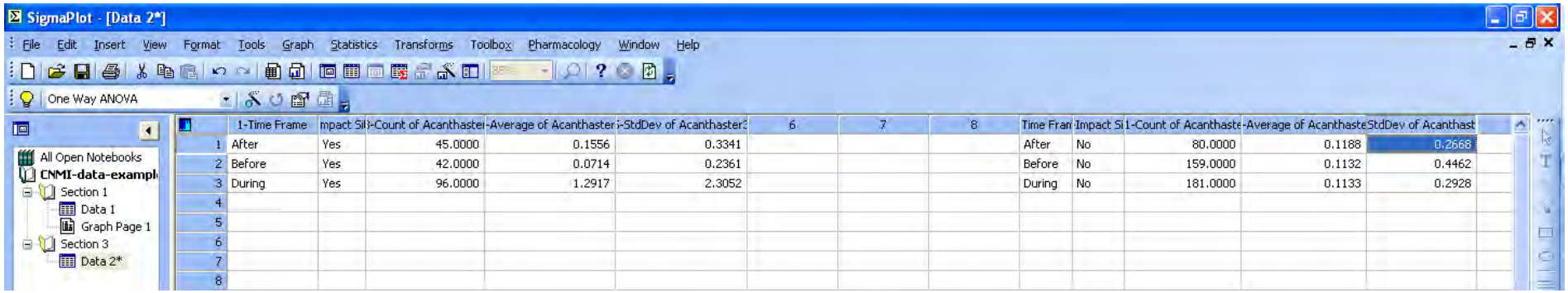


	1-Time Frame	2-Impact Sites	3-Count of Acanthaster	4-Average of Acanthaster2	5-StdDev of Acanthaster3	6
1	After	Yes	45.0000	0.1556	0.3341	
2	Before	Yes	42.0000	0.0714	0.2361	
3	During	Yes	96.0000	1.2917	2.3052	
4						
5						
6						
7						
8						

Now we need to import the data from the sites where COTS abundances showed no increases over the disturbance years.

21. In Excel **change** your "***Impact Sites***" filter to "***No***".
22. **Copy** the entire table.
23. **Go back** to ***Sigma Plot*** (*leave columns 6, 7, and 8 blank - for later use*).
    b. **Right click** on the first cell in ***Column 9*** and **select** "***Paste***".
    c. **Highlight** only the cells you want to include in your data starting with "***COTS***" in the upper left and "***0.2928***" in the lower left (*corresponding to cells 9,2 and 13,5*)
24. **Cut** (*Ctrl + X*) the ***highlighted data***
    a. "Paste" them one row up (***cell 9,1***).
25. **Rename** cell 9,1 from "***COTS***" to "***Time Frame***".

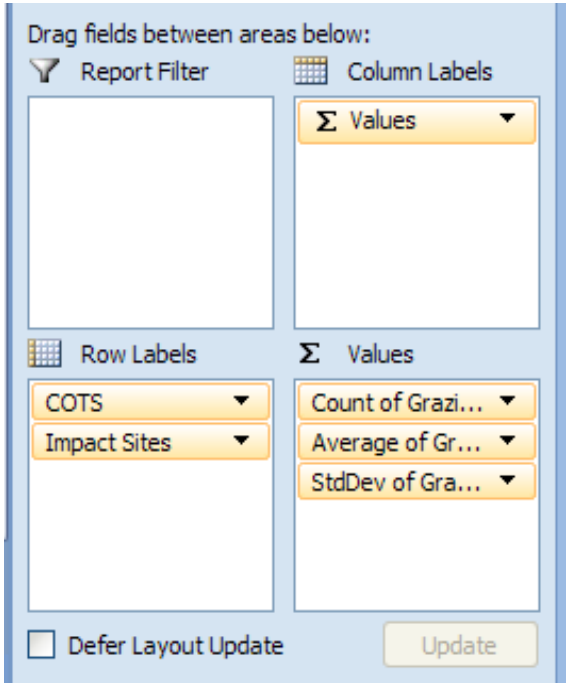
26. **Right click** on ***Column 9***
    a. **Choose** "***Column Titles***"
    b. **Promote** the column headings to titles for all ***5 columns*** (*columns 9-13*).
27. Confirm.



	1-Time Frame	mpact Si	-Count of Acanthaster	-Average of Acanthaster	-StdDev of Acanthaster	6	7	8	Time Fran	Impact Si	1-Count of Acanthaste	-Average of Acanthaste	StdDev of Acanthast
1	After	Yes	45.0000	0.1556	0.3341				After	No	80.0000	0.1188	0.2668
2	Before	Yes	42.0000	0.0714	0.2361				Before	No	159.0000	0.1132	0.4462
3	During	Yes	96.0000	1.2917	2.3052				During	No	181.0000	0.1133	0.2928
4													
5													
6													
7													
8													

Based upon previous exploration of the data using Excel Pivot Tables it was determined that a simultaneous look at grazing sea urchins was most useful to understand some influential trends. We will now place the grazing urchin data alongside the COTS data.

28. **Go back** to Excel.
    a. **Change** the "***Impact Sites***" filter to "***Yes***".
    b. **Remove** all "***Acanthaster***" boxes from under the "***Values***".
29. **Drag** the "***Grazing Urchin Total***" box under "***Values***" ***<u>three times</u>***
    a. **Change** the attributes of the first Grazing Urchin Total box to "***Count***".
    b. **Change** the second to "***Average***".
    c. **Change** the third to "***StdDev***".
30. Confirm.



31. **Copy** the ***relevant data*** cells in Excel.
32. **Return** to Sigma Plot.
    a. **Paste** these data cells below our existing tables (*choose cell 6,10*)
33. Confirm.

	1-Time Frame	mpact Si	-Count of Acanthaster	-Average of Acanthaster	-StdDev of Acanthaster	6	7	8	Time Fran	Impact Si	1-Count of Acanthaste	-Average of Acanthaste	StdDev of Acanthast
1	After	Yes	45.0000	0.1556	0.3341				After	No	80.0000	0.1188	0.2668
2	Before	Yes	42.0000	0.0714	0.2361				Before	No	159.0000	0.1132	0.4462
3	During	Yes	96.0000	1.2917	2.3052				During	No	181.0000	0.1133	0.2928
4													
5													
6													
7													
8													
9													
10						COTS	Impact Sites	Count of Grazing	Average	StdDev o			
11						After	Yes	45.0000	4.7222	3.2710			
12						Before	Yes	42.0000	4.5476	5.0386			
13						During	Yes	96.0000	2.0833	3.0964			
14													

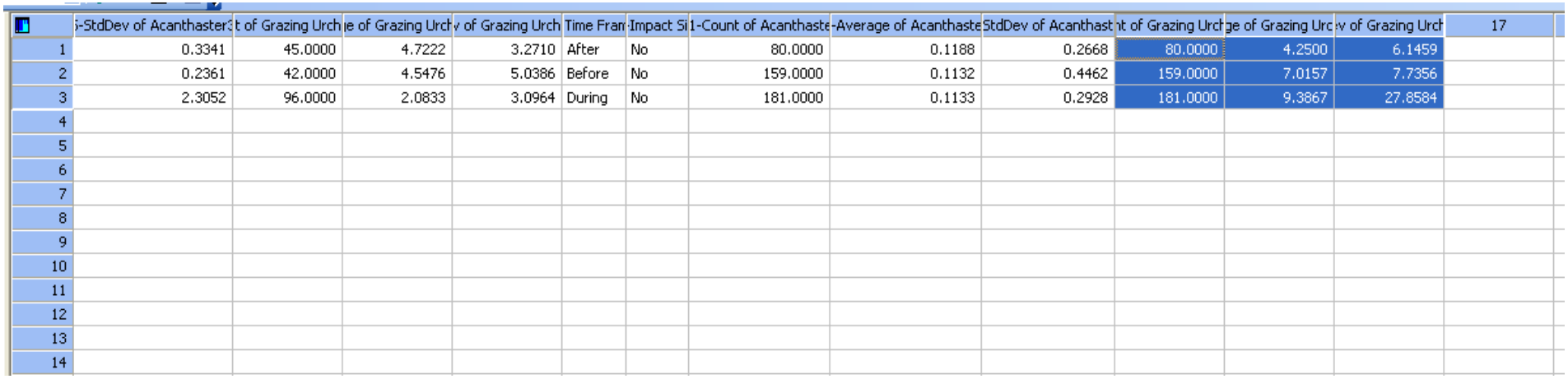
Notice the first two columns are the same and already are presented in columns 1 and 2.

34. **Highlight** just the data, from "***Count of Grazing***" to the number "***3.0964***".
    a. **Cut** and **paste** these data under ***Column 6***.
35. **Promote** the column headings to titles.
    a. **Delete** all unnecessary cells left below.
36. Confirm.

	1-Time Frame	mpact Si	-Count of Acanthaster	-Average of Acanthaster	-StdDev of Acanthaster	t of Grazing Urch	e of Grazing Urch	of Grazing Urch	Time Fran	Impact Si	1-Count of Acanthaste	-Average of Acanthaste	StdDev of Acanthast
1	After	Yes	45.0000	0.1556	0.3341	45.0000	4.7222	3.2710	After	No	80.0000	0.1188	0.2668
2	Before	Yes	42.0000	0.0714	0.2361	42.0000	4.5476	5.0386	Before	No	159.0000	0.1132	0.4462
3	During	Yes	96.0000	1.2917	2.3052	96.0000	2.0833	3.0964	During	No	181.0000	0.1133	0.2928
4													
5													
6													
7													
8													
9													

Finally we get the last set of data from Excel.

37. **Go back** to our ***PivotTable***
    a. **Set** the "***Impact Sites***" filter to "***No***".
38. **Copy** and **paste** these data into Sigma Plot, all the way at the end of our existing table (*i.e., into columns 14, 15, and 16*)
    a. **Promote** the headings to titles
39. Confirm



	-StdDev of Acanthaster	t of Grazing Urch	e of Grazing Urch	v of Grazing Urch	Time Fram	Impact Si	1-Count of Acanthaste	-Average of Acanthaste	StdDev of Acanthast	nt of Grazing Urch	ge of Grazing Urc	v of Grazing Urch	17
1	0.3341	45.0000	4.7222	3.2710	After	No	80.0000	0.1188	0.2668	80.0000	4.2500	6.1459	
2	0.2361	42.0000	4.5476	5.0386	Before	No	159.0000	0.1132	0.4462	159.0000	7.0157	7.7356	
3	2.3052	96.0000	2.0833	3.0964	During	No	181.0000	0.1133	0.2928	181.0000	9.3867	27.8584	
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													

Notice you have to use the lateral scroll bar on the bottom of the screen now as your data exceeds a typical screen view. In the screen shot above scrolling to the right was needed. The highlighted cells show the last data we just brought over.
The last step before proceeding to making graphs is to transform our standard deviations to standard errors that are commonly used for graphical representations of our datasets and understanding statistical significance. Recall that the Standard Error is simply the standard deviation divided by the square root of the sample size.

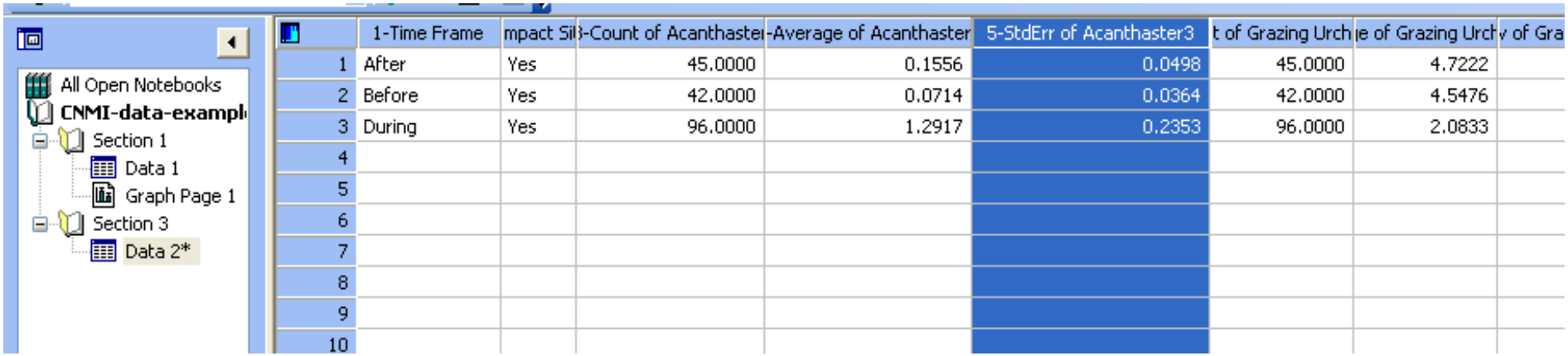
In each instance where a StdDev column of data is present we will change these to StdErr. We have to do this manually as Excel does not have a Standard Error function customized for our needs.

40. **Scroll** to "***StdDev of Acanthaster***" (*Column 5*) associated with the "Yes" impact sites.

Recall that the "Count" column indicates our sample size, so we need to divide the value in "StdDev" cell by the square root of the value in the "Count".

Do this with a calculator, a fresh spreadsheet in Excel, or other means of your choosing.

41. When completed, **replace** the contents of ***Column 5*** with the ***standard errors*** you calculated
    a. **Change** the name of the column from "***StdDev***" to "***StdErr***".
42. Confirm for the first set of data below.



	1-Time Frame	mpact Sit	3-Count of Acanthaster	-Average of Acanthaster	5-StdErr of Acanthaster3	t of Grazing Urch	e of Grazing Urch	y of Gra
1	After	Yes	45.0000	0.1556	0.0498	45.0000	4.7222	
2	Before	Yes	42.0000	0.0714	0.0364	42.0000	4.5476	
3	During	Yes	96.0000	1.2917	0.2353	96.0000	2.0833	
4								
5								
6								
7								
8								
9								
10								

43. Do this for all three other instances where standard deviations existed, so that we have only standard errors showing on our datasheet.

We are now ready to create informational, professional graphs and associated testing using Sigma Plot.
Note: Save your work.

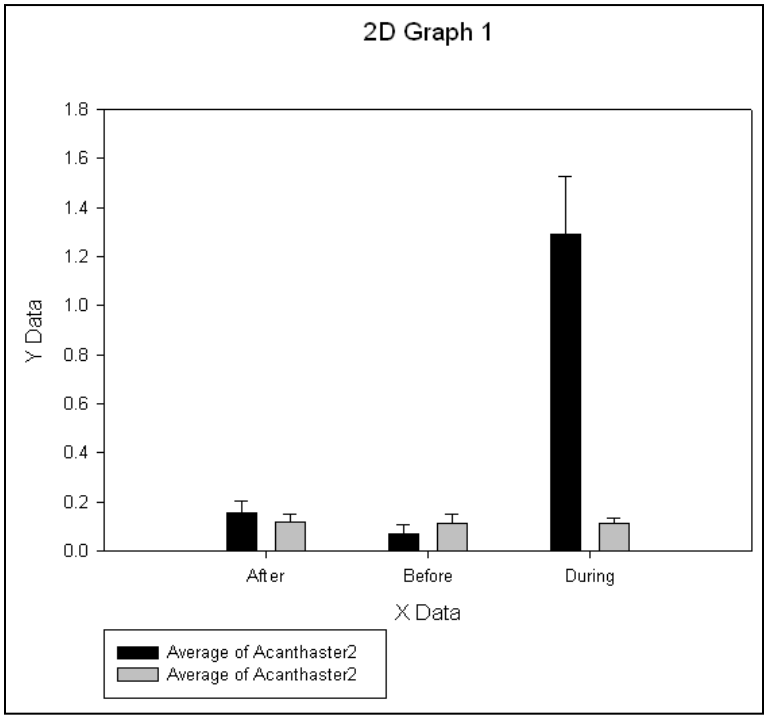
44. **Go to** the "***Graph***" main menu from Sigma Plot and
    a. **Select** "Create Graph".
    b. **Choose** "Vertical Bar Chart".
    c. **Click** next.
45. **Select** "Grouped Error Bars".
    a. **Click** next.
46. For "***Symbol values***"
    a. Make sure "***Worksheet Columns***" is **selected** in the drop down menu.
    b. **Click** next.
47. For ***data format*** choose "***X Many Y***".
    a. **Click** Next.

Now Sigma Plot is ready for our data.

48. For our "***X:***" data
   a. Choose the first column "***Time Frame***".
49. For "***Set 1***:"
   a. Choose "***Average of Acanthaster***" values associated with "***Yes***" impact sites. (*This is column 4*)
50. For "***Error 1***:"
   a. Choose the associated standard errors we just calculated in ***column 5***.

Now were ready to enter a second set of data.

51. For "***Set 2***:"
   a. Choose "***Average of Acanthaster***" values associated with "***No***" impact sites. (*This is column 12*)
52. For "***Error 2***:"
   a. Choose the associated standard errors we just calculated in ***column 13***.
53. Click Finish.
54. When the graph appears **change** the zoom from ***50%*** to ***100%*** in the drop down menu on top of the screen.
55. Confirm.

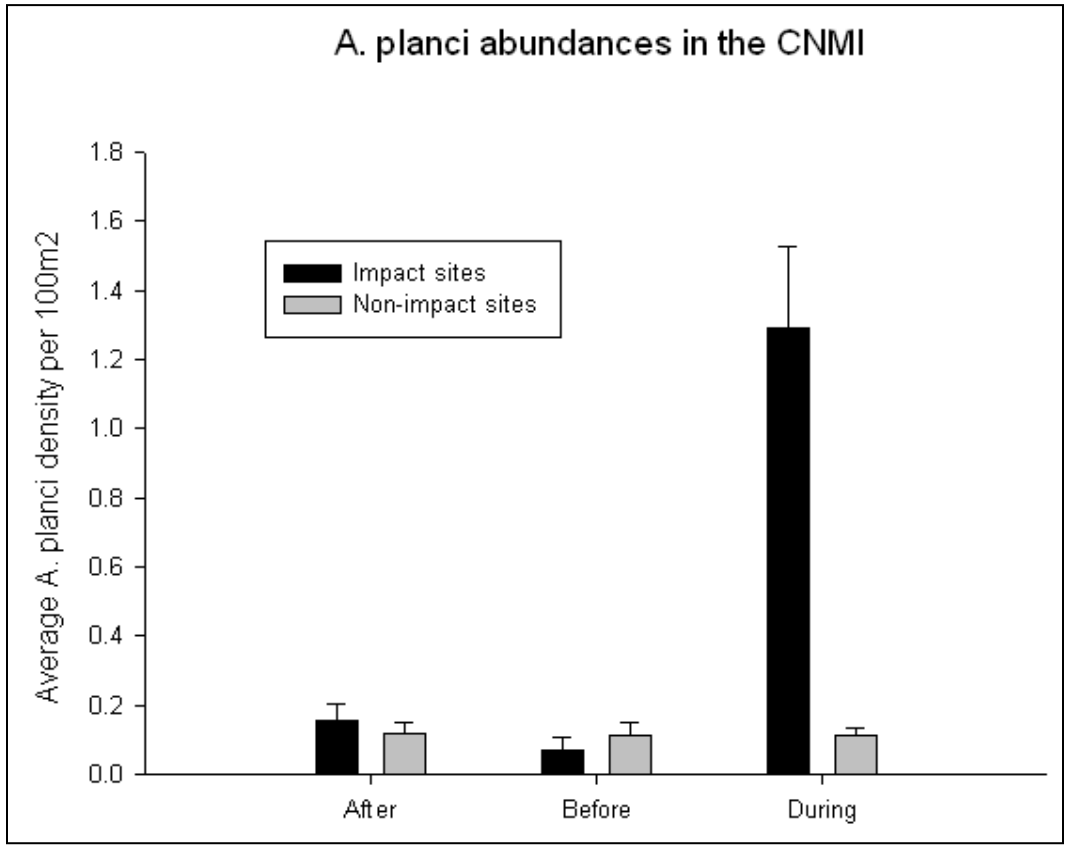


Now some quick changes to our graph appearance.

56. Change the title to "***A. Planci abundances in the CNMI***".
57. Change the "***Y Data***" to "***Average A. Planci density per 100m2***".
58. Delete "***X Data***".
59. In the legend box,
   a. Double click the text next to the ***black box*** and rename it "***Impact sites***".
   b. Double click the text next to the ***grey box*** and rename it "***Non-impact sites***".
60. Move the legend anywhere inside the graph.

Note: You can remove the upper line associated with the graph and the one on the right too if you like, just for appearance.

61. Confirm our new look.



Now we have a very informative graph that is clearly showing a major increase in COTS abundances during the disturbance years at the sites we consider to be "impacted" as compared with all others. Note we can't run a formal statistical analyses on these data because our groupings "impact" or "no impact" were not defined prior to examining the data (or apriori). This is fine because were interested in examining the cascading impacts to the grazing urchins, and eventually graph affinities with coral reef recovery.

62. **Go back** to our Sigma Plot data sheet, "***Data 2***".
63. **Go to** the "***Graph***" main menu from Sigma Plot
    a. **Select** "***Create Graph***".
64. **Choose** "Vertical Bar Chart"
    a. **Click** next.
65. **Select** "Grouped Error Bars".
    a. **Click** next.
66. For "***Symbol values***"
    a. Make sure "***Worksheet Columns***" is **selected** in the drop down menu.
    b. **Click** next.
67. For data format **choose** "***X Many Y***".
    a. **Click** Next.

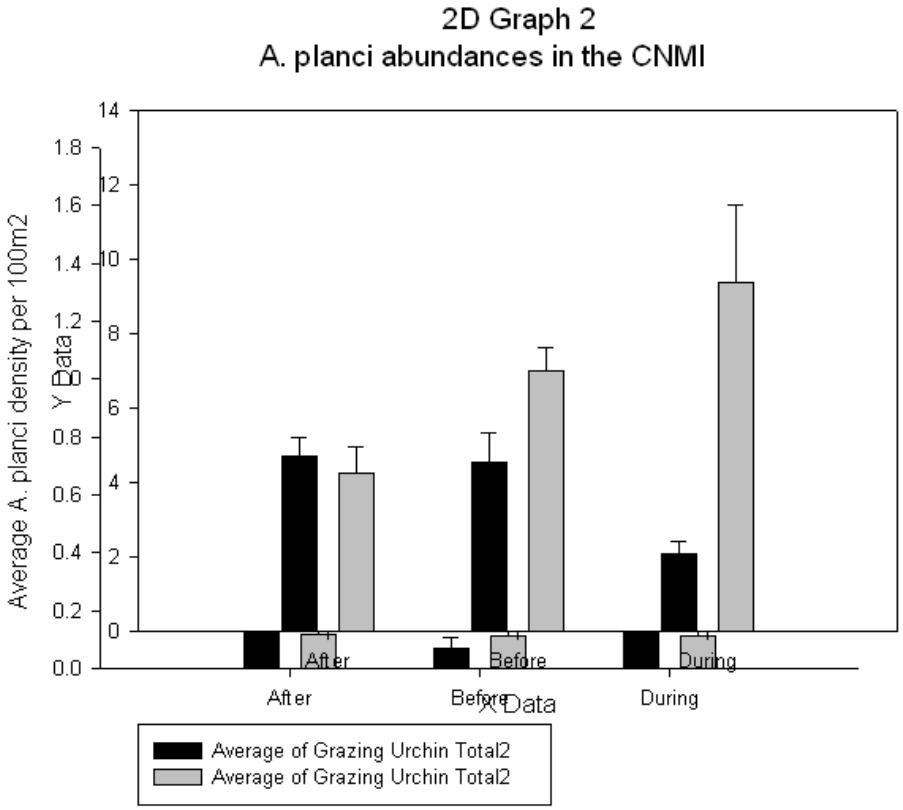
Now Sigma Plot is again ready for our data.

68. For our "***X:***" data **choose** the first column "***Time Frame***".
69. For "***Set 1:***"
    a. **Choose** "***Average of Grazing Urchins***" values associated with "**Yes**" impact sites. (*This is column 7*)
70. For "Error 1:"
    a. **Choose** the associated standard errors we just calculated in ***column 8***.

Now were ready to enter a second set of data.

71. For "***Set 2***:"
    a. **Choose** "***Average of Grazing Urchins***" values associated with "***No***" impact sites. (*This is column 15*)
72. For "Error 2:"
    a. **Choose** the associated standard errors we just calculated in ***column 16***.
73. **Click** Finish.

74. Confirm.



Notice the second graph was created directly on top of our existing graph.
We will first re-arrange our charts.

75. From the zoom drop down menu, **select *50%***.

- **a. Drag** the chart we just created to the bottom of the sheet
- **b. Drag** the ***Acanthaster*** graph to the top.

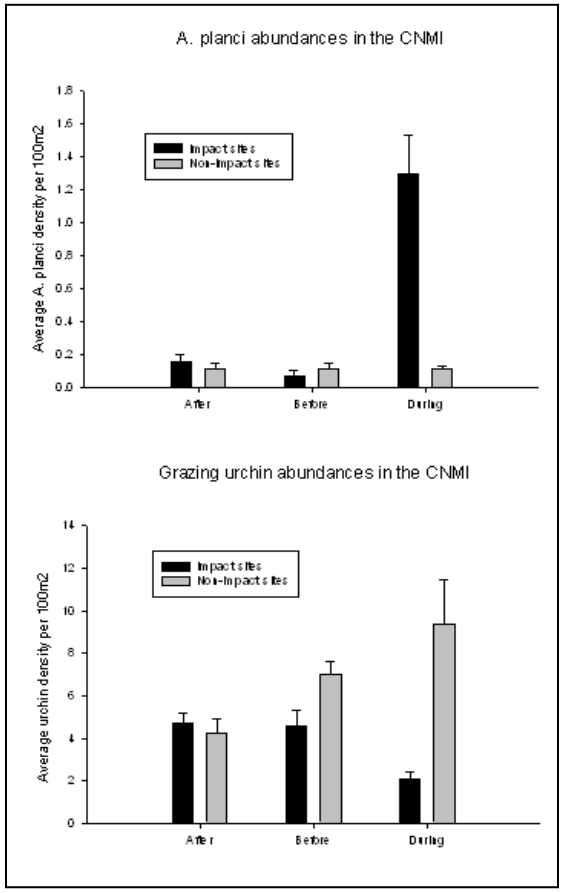
***Note**: Arrange them neatly.*

Now, let's clean up our grazing urchin chart.

76. **Rename** the title to "***Grazing urchin abundances in the CNMI***".
77. **Rename** the "***Y Data***" to "***Average urchin density per 100m2***".
78. **Delete** "**X Data**".
79. In the Legend
    a. **Double click** the text next to the ***black box*** and **rename** it to "***Impact sites***".
    b. **Double click** the text next to the ***grey box*** and **rename** it to "***Non-impact sites***".
80. **Drag** the legend anywhere inside the graph.

Note*: You can remove the upper line associated with the graph and the one on the right too if you like, just for appearance.*

81. **Change** the zoom drop down menu to "***Fit***".
82. Confirm our new look of the two graphs.



Consider these very interesting results. At the impact sites where COTS abundances were high we have noted what seems to be a significant decline in grazing urchins. It appears that when the COTS abundances grew, the urchin abundances declined. Strong evidence comes from the fact that the trend was only noted at the impact sites. We know how important grazing urchins are for reefs to recover, so the findings are clearly influential. What we don't know is how the declines in urchins occurred. Are Acanthaster superior to the grazing urchins and able to take all of the good hiding spots in the reef, leaving the urchins open for predation? Was there a direct competitive interaction? We don't know the answers to these questions, but the trend we do know is of great concern. Let's see if these findings are indeed significant.

Sigma Plot has a number of built in statistical testing procedures. We will use a straightforward ANOVA test to examine if there were differences in urchin densities between the timeframes, at both the "Impact" and "Non-impact" sites. ANOVA tests compare the distributions of the samples, and require us to input means, standard errors, and sample sizes for each set of measurements. This guidebook assumes you have basic statistical knowledge; however any introductory statistics book can serve as a guide to better understand the procedures available in Sigma Plot. There is also a well developed "Help" menu with lots of additional information.

83. Click back on our "***Data 2***" sheet.

First we will analyze if urchins densities from the impact sites were significantly different during each time frame.

84. Under the "***View***" main menu
- **a. Scroll down** to "***Toolbars***" and make sure "***Statistics***" is highlighted.

You should see a statistics toolbar appear, it has a yellow light bulb icon and a drop down menu next to it.

85. In the ***drop down menu***
- **a. Scroll down** to "***One Way ANOVA***".
- **b. Click** on the ***magic wand icon*** next to the drop down menu.

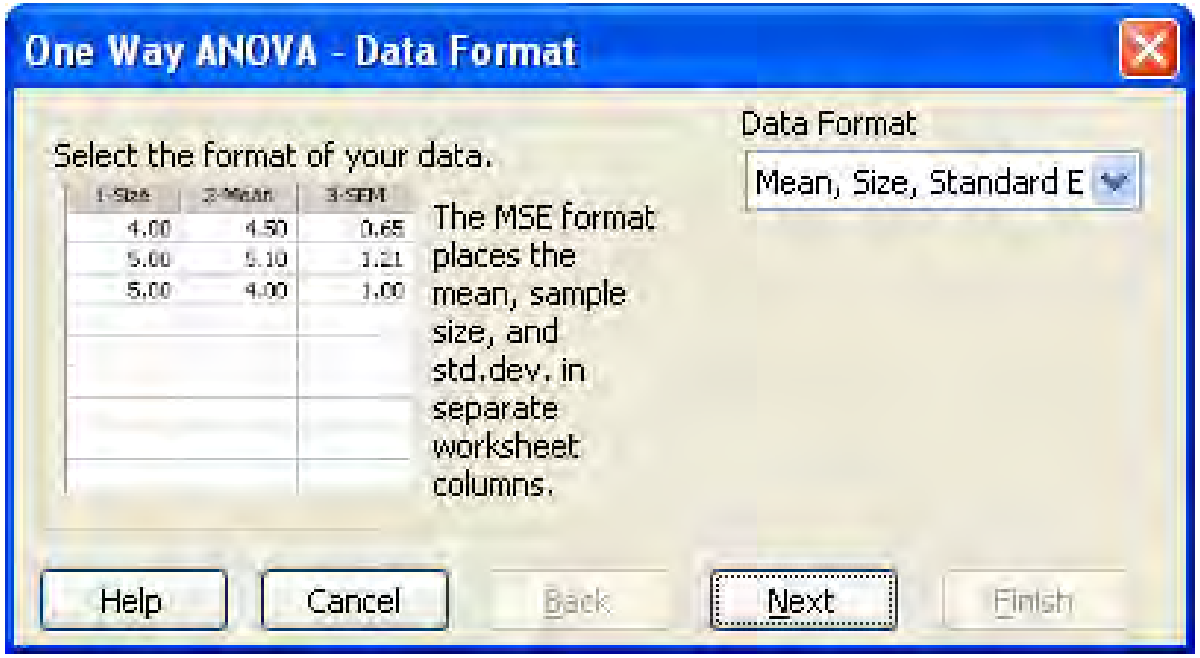
The first step is to define our data format.

- **c. Select** "***Mean***, ***Size***, ***Standard Error***" to match our data.

86. Click next.

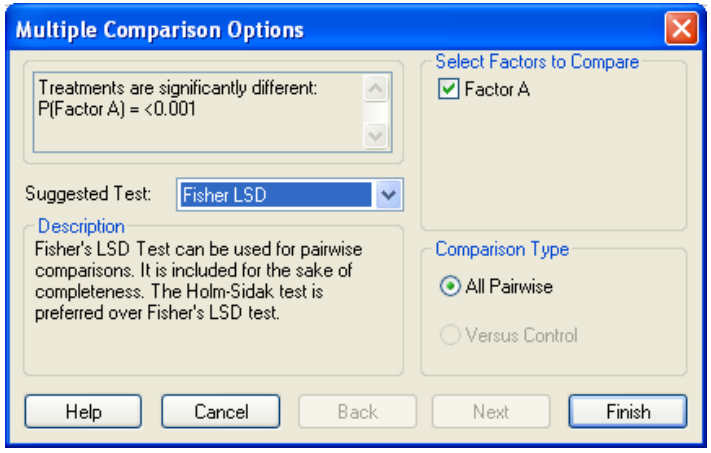
Now we are asked for our dataset. First we will test whether or not grazing urchin abundances differed at the “Impact” sites during the different time periods.

87. When asked for our “***Data for Mean:***”,
   a. Choose column 7, or “***Average of Grazing Urchin***” (*which corresponds to average abundances within our impact sites*)
88. When asked for our “***Size***:” (*remember this is sample size*)
   a. Select our ***Count data*** located in column 6.
89. When asked for ***Standard Error***
   a. Choose *column 8*.
90. Click Finish.
91. Confirm.



The informational box tells us that “Treatments are significantly different”, meaning that urchin abundances are significantly different among the time frames at the impact sites.
We need to know which time frames are different from each other because there are three. So the dialog box asks us logically to choose a comparison of individual means.

92. Select "Fisher LSD" from the drop down menu.



This is one popular post-hoc comparison of means test used in ecology.

93. Click Finish.

94. Confirm the statistical testing results sheet below.

One Way Analysis of Variance Monday, June 28, 2010, 12:43:06 PM

Data source: Data 2 in CNMI-data-example.JNB

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	45	0	4.722	3.271	0.488
Row 2	42	0	4.548	5.039	0.777
Row 3	96	0	2.083	3.096	0.316

Source of Variation	DF	SS	MS	F	P
Between Groups	2	298.503	149.252	11.090	<0.001
Residual	180	2422.493	13.458		
Total	182	2720.997			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.991

All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor:

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
Row 1 vs. Row 3	2.639	1.308	<0.001	Yes
Row 1 vs. Row 2	0.175	1.553	0.825	No
Row 2 vs. Row 3	2.464	1.339	<0.001	Yes

Note on this sheet the groups are referred to as Row 1, 2, and 3. From our main data sheet we know that Row 1 represents the time frame after the COTS disturbances, Row 2 is before, and Row 3 is during. General data summaries that we selected for input first appear under "Group Name". Then under "Source of Variation" we have our ANOVA table showing significant differences between the groups (but we don't yet know which ones, just that variation exists). Finally, under "Comparison" we see individual pairwise testing results. Pairwise testing shows that Row 1 is unique and significantly different from all others, and Row's 2 and 3 are the same. Translated, urchin densities significantly declined during the years where A. planci abundances were high, but seem to have rebounded.

We will now look at the "Non-impact" sites where we hypothesize that no change in urchin densities would have occurred.

95. Click on the magic wand icon next to the drop down menu.
Again, the first step is to define our data format.

   a. **Select** "Mean, Size, Standard Error" to match our data.
   b. **Click** next.

96. For "***Data for Mean***:",
   a. **Select** column 15, or "***Average of Grazing Urchin***" (*which corresponds to average abundances within our Non-impact sites*)

97. For our "***Size***:" (*remember this is sample size*)
   a. **Select *Count data*** located in column 14.

98. For ***Standard Error***
   a. **Select** column 16.

99. Click Finish.

100. Confirm.

One Way Analysis of Variance Monday, June 28, 2010, 4:19:14 PM

Data source: Data 2 in CNMI-data-example.JNB

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	80	0	4.250	6.146	0.687
Row 2	159	0	7.016	7.736	0.613
Row 3	181	0	9.387	27.858	2.071

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1526.563	763.281	2.092	0.125
Residual	417	152134.918	364.832		
Total	419	153661.481			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.125).

Power of performed test with alpha = 0.050: 0.231

The power of the performed test (0.231) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists.
Negative results should be interpreted cautiously.

The resultant summary sheet informs us that no significant variation was detected. We can look under the "Source of Variation" section and see our P-value is much greater than 0.05, typically required for significance. Thus, there is no need to conduct pairwise testing because no overall significant variation was detected. This tells us that at sites where no major increases in A. planci density were observed the urchin density remained the same. We can now be pretty confident in our conclusions that are graphically represented.

We are completed with this exercise, however you can open another existing file to better understand the ecological consequences associated with high A. planci densities in the CNMI from 2003-2006.

End of Exercise 6.2

Section 3 – Multivariate statistics and graphing the results

Exercise 7 – An introduction to multivariate data considerations, PRIMER-E, and PERMANOVA+

For this exercise we will again consider fish biomass data collected along replicate transect lines, this time from Nimpal and Gachuug municipalities, Yap State, Federated States of Micronesia. Rather than focus upon any individual species of fish, or compare "total biomass", we will now begin to address the multivariate nature that many ecological datasets have. Yap Community Action Program's marine office conducts monitoring at several localities that desire to establish Marine Protected Areas (MPA) for conservation purposes. Similar to Pohnpei, for each MPA that is surveyed, there is an ecologically-similar reference site. Yap's program collects data at two different depths, a 3m and 10m.

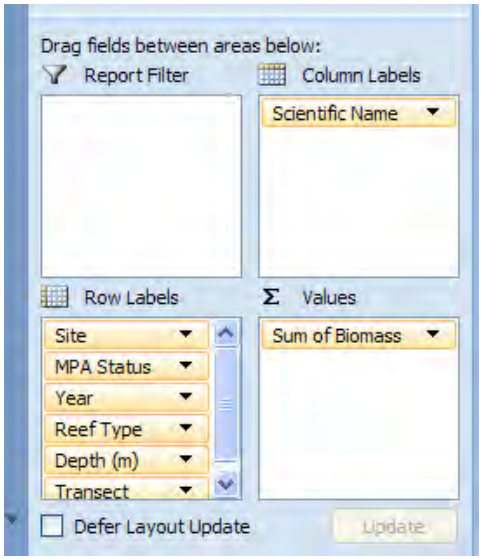
1. **Open** Excel
   a. **Open** "***Yap-Nimpal-MPA-Fish***".

*Notice the database, site metadata, and fish species lookup tables that were used to generate the database. In this database each row represents **one or more fish** of the **exact same size**, of a particular species, that was observed on **a single** transect. This structure represents another commonly used format for reporting upon visual census fish data. For the Pohnpei database recall that each row was one individual fish only, here **column J** indicates how many fish were seen on any transect of the same species and size. Make sure you understand that before moving forward.*

We will need to prepare a table that we can import to PRIMER-E for further analyses. We'll again use Pivot Table features.

2. **Highlight** the data and **insert** a Pivot Table,
   a. Name the table "Yap-fish-pivot".
   b. **Add** "***Site***", "***MPA Status***", "***Year***", "***Reef Type***", "***Depth (m)***", and "***Transect***" all under Row Labels (*in that order*)
   c. **Add** "***Scientific Name***" to the Column Labels.
   d. **Add** "***Biomass***" to the Values.
   e. **Change** the attributes of Biomass to "***Sum***".

3. Confirm.



Now modify the way the table looks for easiest input into PRIMER.

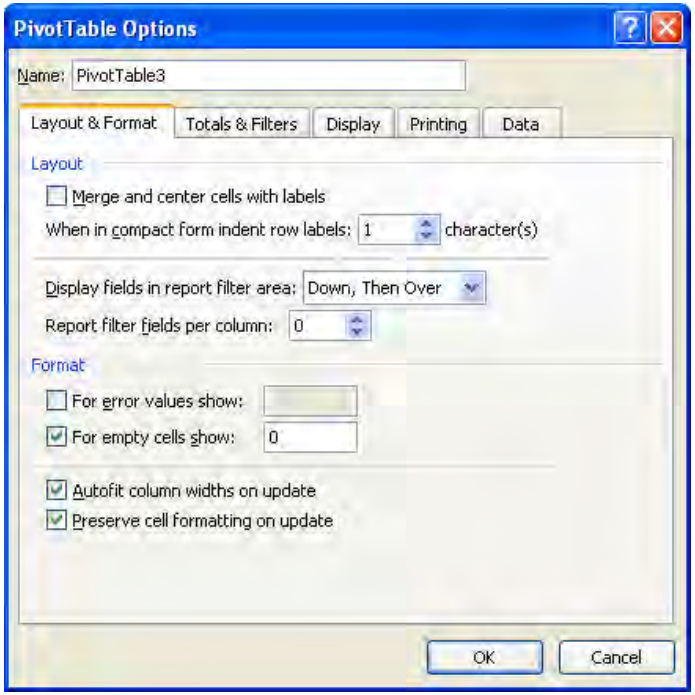
4. **Right click** in cell ***A5***
   a. **Select** "***Field Settings***",
   b. Under "Subtotals", **select** "***None***".
5. Confirm.

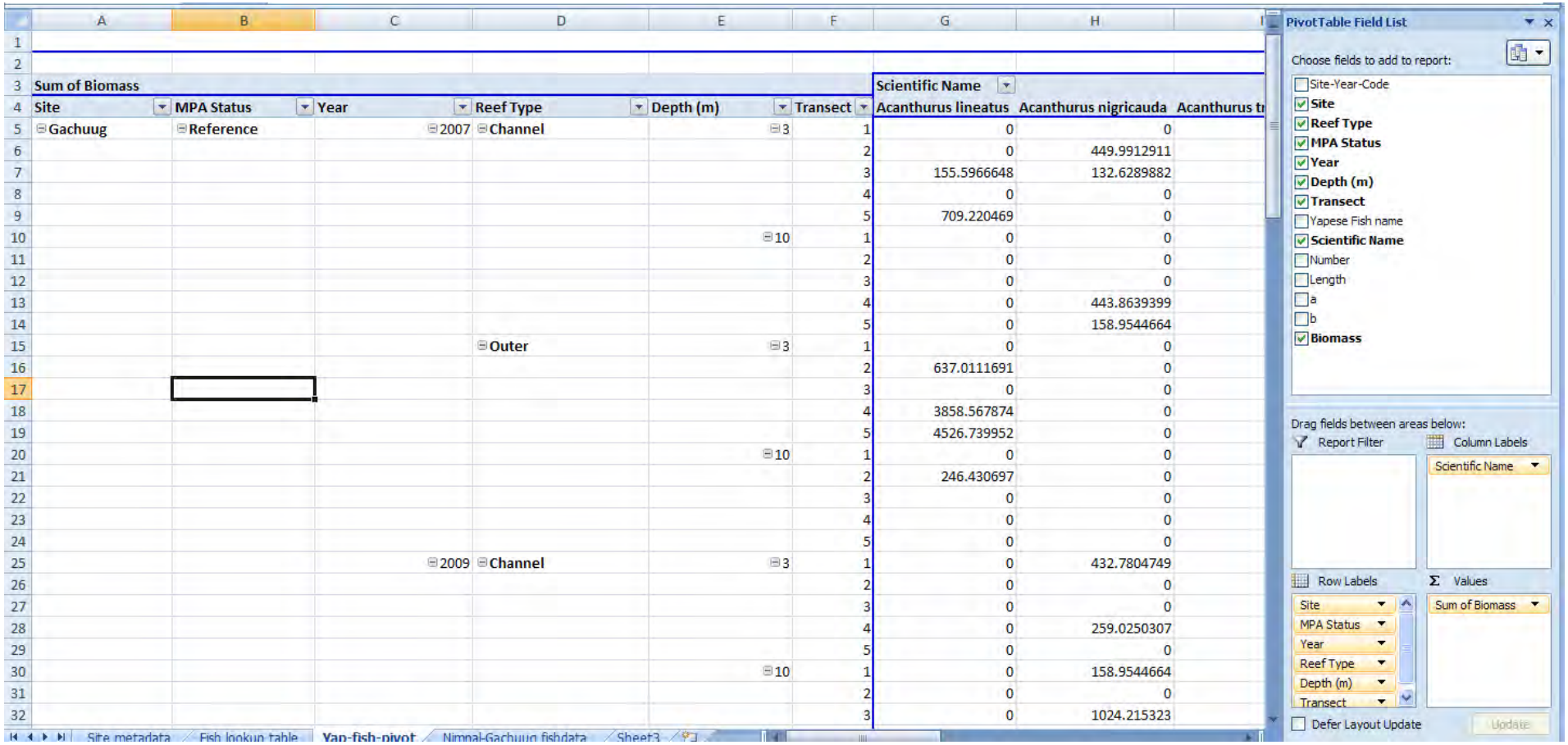
6. **Repeat** for cells A6, A7, A8, and A9.

This will condense all of the subtotals that Excel auto-generates.

7. **Right click** anywhere in the table
   a. **Scroll down** and **select** "***PivotTable Options***".
8. On the tab "***Layout & Format***"
   a. **Check** the box that says "***For empty cells show***:"
   b. **Put** a "***0***" in the box.
9. On the tab "Totals & Filters"
   a. **Uncheck** the box "***grand totals for rows and columns***".
10. On the tab "***Display***"
    a. **Check** the box "***Classic Pivot Table layout***".

11. Confirm these settings and the new table look.



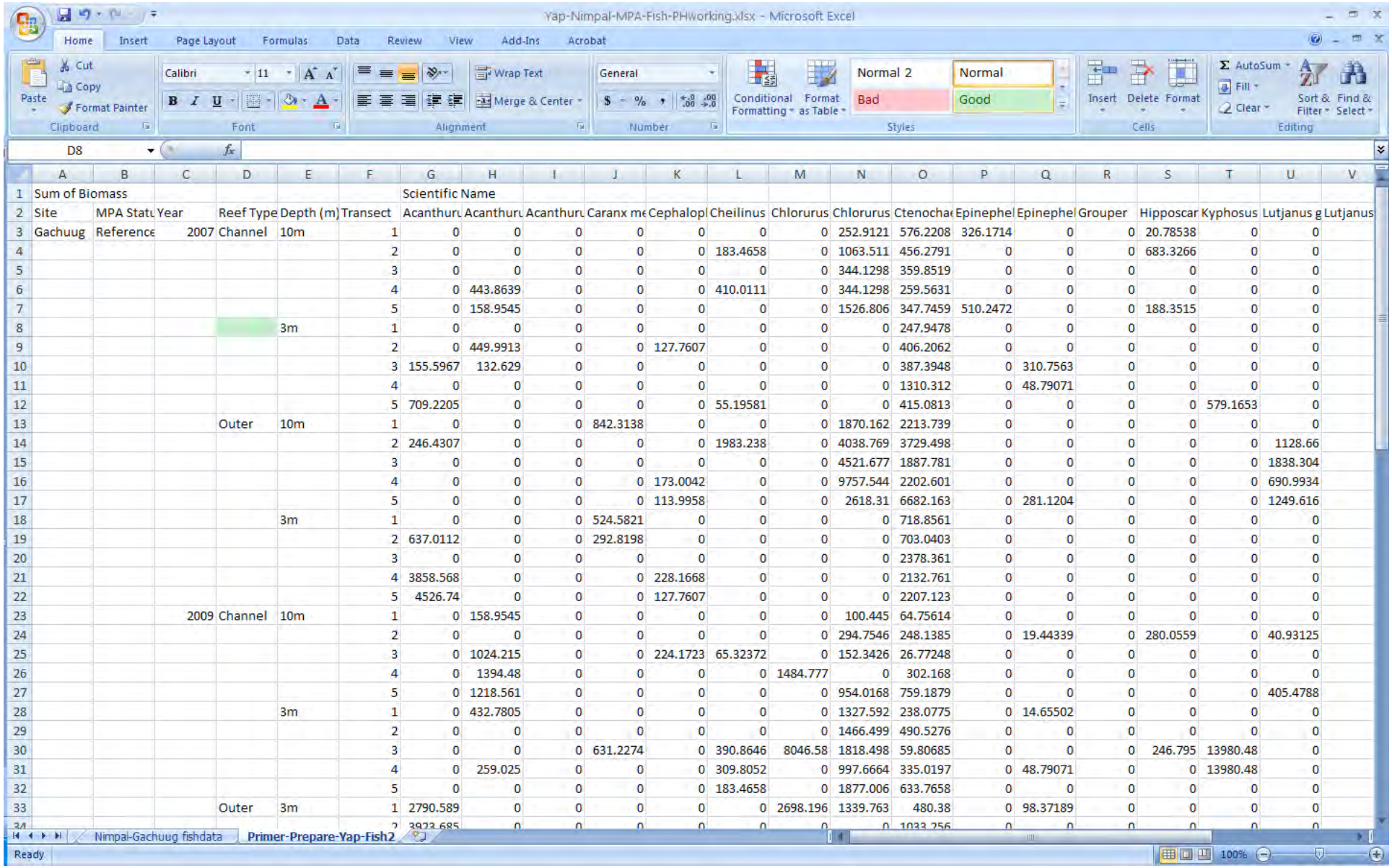


Sum of Biomass						Scientific Name		
Site	MPA Status	Year	Reef Type	Depth (m)	Transect	Acanthurus lineatus	Acanthurus nigricauda	Acanthurus t
Gachuug	Reference	2007	Channel	3	1	0	0	
					2	0	449.9912911	
					3	155.5966648	132.6289882	
					4	0	0	
					5	709.220469	0	
				10	1	0	0	
					2	0	0	
					3	0	0	
					4	0	443.8639399	
					5	0	158.9544664	
			Outer	3	1	0	0	
					2	637.0111691	0	
					3	0	0	
					4	3858.567874	0	
					5	4526.739952	0	
				10	1	0	0	
					2	246.430697	0	
					3	0	0	
					4	0	0	
					5	0	0	
		2009	Channel	3	1	0	432.7804749	
					2	0	0	
					3	0	0	
					4	0	259.0250307	
					5	0	0	
				10	1	0	158.9544664	
					2	0	0	
					3	0	1024.215323	

We will export this to a new sheet now.

12. **Click** on any cell in the table.
    a. **Press *Ctrl+A***.
    b. **Right click** and **select** "***Copy***".
13. **Select** "Sheet3".
    a. **Right click** in cell A1
    b. **Select** "Paste Special" and **select** "Values".
    c. **Click** OK.
14. **Rename** the sheet "***Primer-Prepare-Yap-Fish***".

15. Confirm.



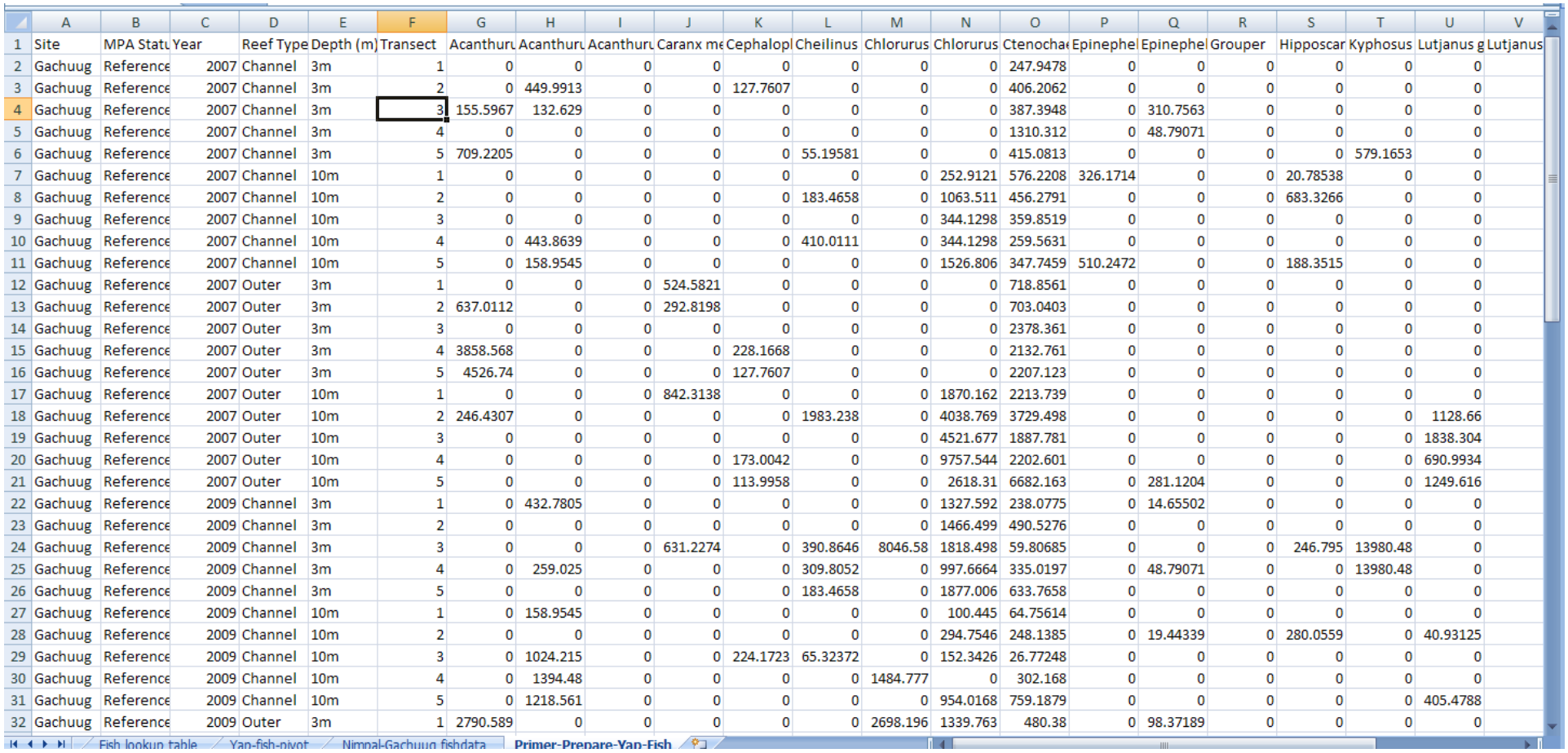
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Sum of Biomass						Scientific Name															
2	Site	MPA Statu	Year	Reef Type	Depth (m)	Transect	Acanthuru	Acanthuru	Acanthuru	Caranx me	Cephalopl	Cheilinus	Chlorurus	Chlorurus	Ctenochae	Epinephel	Epinephel	Grouper	Hipposcar	Kyphosus	Lutjanus g	Lutjanus
3	Gachuug	Reference	2007	Channel	10m	1	0	0	0	0	0	0	0	252.9121	576.2208	326.1714	0	0	20.78538	0	0	
4						2	0	0	0	0	0	183.4658	0	1063.511	456.2791	0	0	0	683.3266	0	0	
5						3	0	0	0	0	0	0	0	344.1298	359.8519	0	0	0	0	0	0	
6						4	0	443.8639	0	0	0	410.0111	0	344.1298	259.5631	0	0	0	0	0	0	
7						5	0	158.9545	0	0	0	0	0	1526.806	347.7459	510.2472	0	0	188.3515	0	0	
8					3m	1	0	0	0	0	0	0	0	0	247.9478	0	0	0	0	0	0	
9						2	0	449.9913	0	0	127.7607	0	0	0	406.2062	0	0	0	0	0	0	
10						3	155.5967	132.629	0	0	0	0	0	0	387.3948	0	310.7563	0	0	0	0	
11						4	0	0	0	0	0	0	0	0	1310.312	0	48.79071	0	0	0	0	
12						5	709.2205	0	0	0	0	55.19581	0	0	415.0813	0	0	0	0	579.1653	0	
13				Outer	10m	1	0	0	0	842.3138	0	0	0	1870.162	2213.739	0	0	0	0	0	0	
14						2	246.4307	0	0	0	0	1983.238	0	4038.769	3729.498	0	0	0	0	0	1128.66	
15						3	0	0	0	0	0	0	0	4521.677	1887.781	0	0	0	0	0	1838.304	
16						4	0	0	0	0	173.0042	0	0	9757.544	2202.601	0	0	0	0	0	690.9934	
17						5	0	0	0	0	113.9958	0	0	2618.31	6682.163	0	281.1204	0	0	0	1249.616	
18					3m	1	0	0	0	524.5821	0	0	0	0	718.8561	0	0	0	0	0	0	
19						2	637.0112	0	0	292.8198	0	0	0	0	703.0403	0	0	0	0	0	0	
20						3	0	0	0	0	0	0	0	0	2378.361	0	0	0	0	0	0	
21						4	3858.568	0	0	0	228.1668	0	0	0	2132.761	0	0	0	0	0	0	
22						5	4526.74	0	0	0	127.7607	0	0	0	2207.123	0	0	0	0	0	0	
23			2009	Channel	10m	1	0	158.9545	0	0	0	0	0	100.445	64.75614	0	0	0	0	0	0	
24						2	0	0	0	0	0	0	0	294.7546	248.1385	0	19.44339	0	280.0559	0	40.93125	
25						3	0	1024.215	0	0	224.1723	65.32372	0	152.3426	26.77248	0	0	0	0	0	0	
26						4	0	1394.48	0	0	0	0	1484.777	0	302.168	0	0	0	0	0	0	
27						5	0	1218.561	0	0	0	0	0	954.0168	759.1879	0	0	0	0	0	405.4788	
28					3m	1	0	432.7805	0	0	0	0	0	1327.592	238.0775	0	14.65502	0	0	0	0	
29						2	0	0	0	0	0	0	0	1466.499	490.5276	0	0	0	0	0	0	
30						3	0	0	0	631.2274	0	390.8646	8046.58	1818.498	59.80685	0	0	0	246.795	13980.48	0	
31						4	0	259.025	0	0	0	309.8052	0	997.6664	335.0197	0	48.79071	0	0	13980.48	0	
32						5	0	0	0	0	0	183.4658	0	1877.006	633.7658	0	0	0	0	0	0	
33				Outer	3m	1	2790.589	0	0	0	0	0	2698.196	1339.763	480.38	0	98.37189	0	0	0	0	
34						2	3923.685	0	0	0	0	0	0	0	1033.256	0	0	0	0	0	0	

16. Delete extraneous rows and columns.

- **a. Delete** Row ***1***.
- **b. Delete** Row ***77***

c. **Delete** column "***AC***".

Now, we have to fill in the missing cells in columns A through E, with fill down functions similar to before. Do this on your own and confirm the look of your working data table below.



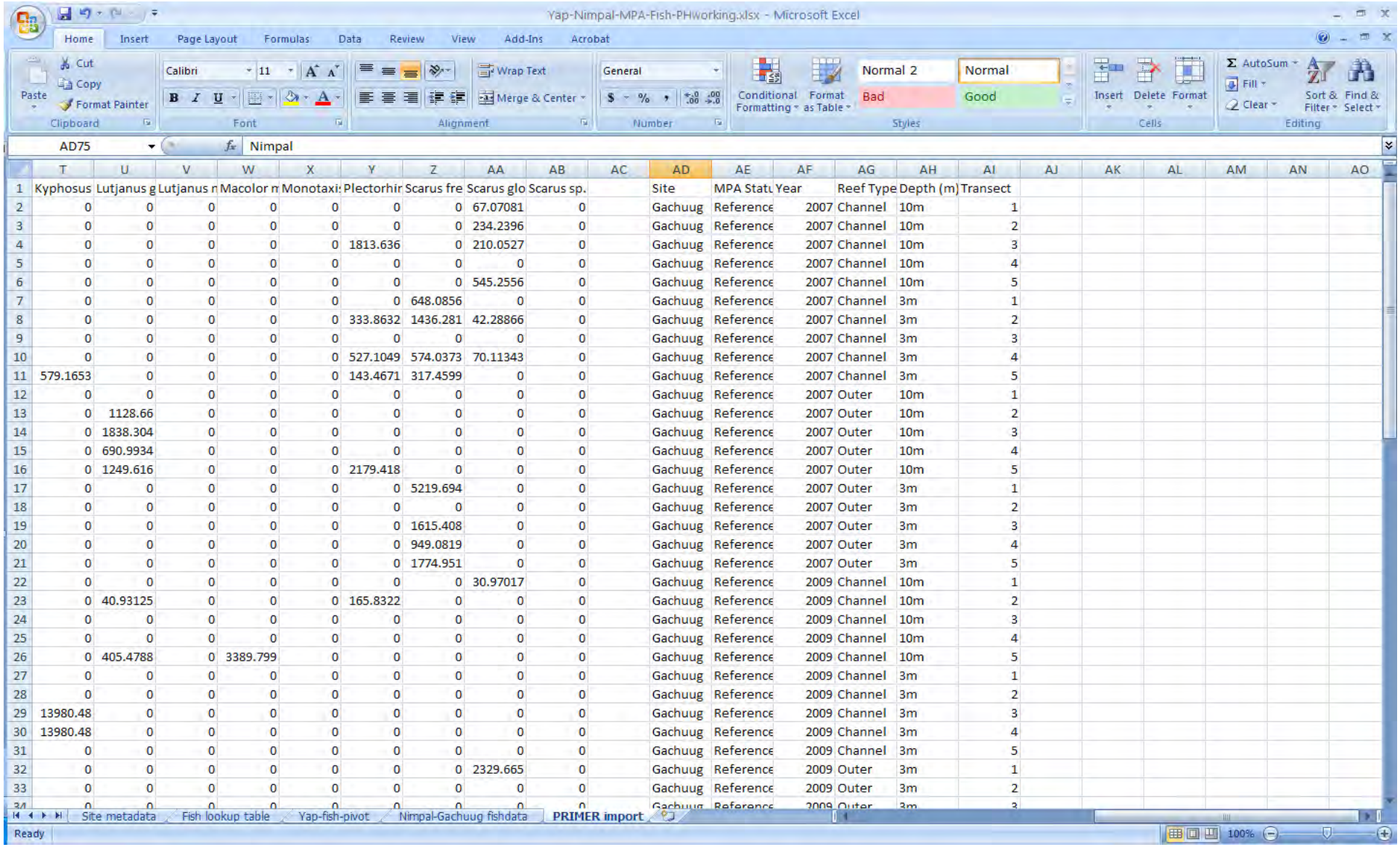
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Site	MPA Statu	Year	Reef Type	Depth (m)	Transect	Acanthuru	Acanthuru	Acanthuru	Caranx me	Cephalopl	Cheilinus	Chlorurus	Chlorurus	Ctenocha	Epinephel	Epinephel	Grouper	Hipposcar	Kyphosus	Lutjanus g	Lutjanus
2	Gachuug	Reference	2007	Channel	3m	1	0	0	0	0	0	0	0	0	247.9478	0	0	0	0	0	0	
3	Gachuug	Reference	2007	Channel	3m	2	0	449.9913	0	0	127.7607	0	0	0	406.2062	0	0	0	0	0	0	
4	Gachuug	Reference	2007	Channel	3m	3	155.5967	132.629	0	0	0	0	0	0	387.3948	0	310.7563	0	0	0	0	
5	Gachuug	Reference	2007	Channel	3m	4	0	0	0	0	0	0	0	0	1310.312	0	48.79071	0	0	0	0	
6	Gachuug	Reference	2007	Channel	3m	5	709.2205	0	0	0	0	55.19581	0	0	415.0813	0	0	0	0	579.1653	0	
7	Gachuug	Reference	2007	Channel	10m	1	0	0	0	0	0	0	0	252.9121	576.2208	326.1714	0	0	20.78538	0	0	
8	Gachuug	Reference	2007	Channel	10m	2	0	0	0	0	0	183.4658	0	1063.511	456.2791	0	0	0	683.3266	0	0	
9	Gachuug	Reference	2007	Channel	10m	3	0	0	0	0	0	0	0	344.1298	359.8519	0	0	0	0	0	0	
10	Gachuug	Reference	2007	Channel	10m	4	0	443.8639	0	0	0	410.0111	0	344.1298	259.5631	0	0	0	0	0	0	
11	Gachuug	Reference	2007	Channel	10m	5	0	158.9545	0	0	0	0	0	1526.806	347.7459	510.2472	0	0	188.3515	0	0	
12	Gachuug	Reference	2007	Outer	3m	1	0	0	0	524.5821	0	0	0	0	718.8561	0	0	0	0	0	0	
13	Gachuug	Reference	2007	Outer	3m	2	637.0112	0	0	292.8198	0	0	0	0	703.0403	0	0	0	0	0	0	
14	Gachuug	Reference	2007	Outer	3m	3	0	0	0	0	0	0	0	0	2378.361	0	0	0	0	0	0	
15	Gachuug	Reference	2007	Outer	3m	4	3858.568	0	0	0	228.1668	0	0	0	2132.761	0	0	0	0	0	0	
16	Gachuug	Reference	2007	Outer	3m	5	4526.74	0	0	0	127.7607	0	0	0	2207.123	0	0	0	0	0	0	
17	Gachuug	Reference	2007	Outer	10m	1	0	0	0	842.3138	0	0	0	1870.162	2213.739	0	0	0	0	0	0	
18	Gachuug	Reference	2007	Outer	10m	2	246.4307	0	0	0	0	1983.238	0	4038.769	3729.498	0	0	0	0	0	1128.66	
19	Gachuug	Reference	2007	Outer	10m	3	0	0	0	0	0	0	0	4521.677	1887.781	0	0	0	0	0	1838.304	
20	Gachuug	Reference	2007	Outer	10m	4	0	0	0	0	173.0042	0	0	9757.544	2202.601	0	0	0	0	0	690.9934	
21	Gachuug	Reference	2007	Outer	10m	5	0	0	0	0	113.9958	0	0	2618.31	6682.163	0	281.1204	0	0	0	1249.616	
22	Gachuug	Reference	2009	Channel	3m	1	0	432.7805	0	0	0	0	0	1327.592	238.0775	0	14.65502	0	0	0	0	
23	Gachuug	Reference	2009	Channel	3m	2	0	0	0	0	0	0	0	1466.499	490.5276	0	0	0	0	0	0	
24	Gachuug	Reference	2009	Channel	3m	3	0	0	0	631.2274	0	390.8646	8046.58	1818.498	59.80685	0	0	0	246.795	13980.48	0	
25	Gachuug	Reference	2009	Channel	3m	4	0	259.025	0	0	0	309.8052	0	997.6664	335.0197	0	48.79071	0	0	13980.48	0	
26	Gachuug	Reference	2009	Channel	3m	5	0	0	0	0	0	183.4658	0	1877.006	633.7658	0	0	0	0	0	0	
27	Gachuug	Reference	2009	Channel	10m	1	0	158.9545	0	0	0	0	0	100.445	64.75614	0	0	0	0	0	0	
28	Gachuug	Reference	2009	Channel	10m	2	0	0	0	0	0	0	0	294.7546	248.1385	0	19.44339	0	280.0559	0	40.93125	
29	Gachuug	Reference	2009	Channel	10m	3	0	1024.215	0	0	224.1723	65.32372	0	152.3426	26.77248	0	0	0	0	0	0	
30	Gachuug	Reference	2009	Channel	10m	4	0	1394.48	0	0	0	0	1484.777	0	302.168	0	0	0	0	0	0	
31	Gachuug	Reference	2009	Channel	10m	5	0	1218.561	0	0	0	0	0	954.0168	759.1879	0	0	0	0	0	405.4788	
32	Gachuug	Reference	2009	Outer	3m	1	2790.589	0	0	0	0	0	2698.196	1339.763	480.38	0	98.37189	0	0	0	0	

Fish lookup table | Yap-fish-pivot | Nimpal-Gachuug fishdata | Primer-Prepare-Yap-Fish

We have one last step before we can import our file into PRIMER-E. We must distinguish between the metadata and the ecological data. PRIMER-E requires that all data appear first, followed by a blank column, then the metadata. We will re-format accordingly.

17. **Highlight** columns A through F
18. **Right click** the highlighted cells and **select** cut.

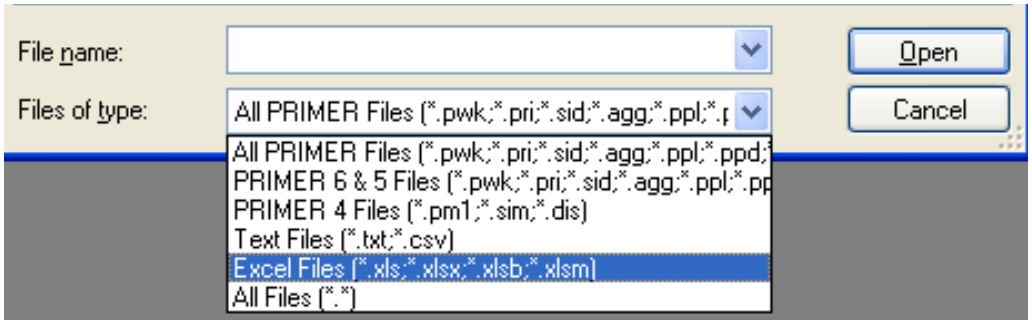
19. **Scroll** to the right of your sheet until you get to column AD
    a. **Right click** in cell AD1, and select **Paste**
    b. **Rename** this sheet now to "***PRIMER import***"
    c. **Scroll** back to columns A to F and note they are blank. **Delete** all of these columns.
    d. Confirm – note you can only see the right side of the database in this screen shot, where we moved the metadata



	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO
1	Kyphosus	Lutjanus g	Lutjanus n	Macolor n	Monotaxi	Plectorhir	Scarus fre	Scarus glo	Scarus sp.		Site	MPA Statu	Year	Reef Type	Depth (m)	Transect						
2	0	0	0	0	0	0	0	67.07081	0		Gachuug	Reference	2007	Channel	10m	1						
3	0	0	0	0	0	0	0	234.2396	0		Gachuug	Reference	2007	Channel	10m	2						
4	0	0	0	0	0	1813.636	0	210.0527	0		Gachuug	Reference	2007	Channel	10m	3						
5	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2007	Channel	10m	4						
6	0	0	0	0	0	0	0	545.2556	0		Gachuug	Reference	2007	Channel	10m	5						
7	0	0	0	0	0	0	648.0856	0	0		Gachuug	Reference	2007	Channel	3m	1						
8	0	0	0	0	0	333.8632	1436.281	42.28866	0		Gachuug	Reference	2007	Channel	3m	2						
9	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2007	Channel	3m	3						
10	0	0	0	0	0	527.1049	574.0373	70.11343	0		Gachuug	Reference	2007	Channel	3m	4						
11	579.1653	0	0	0	0	143.4671	317.4599	0	0		Gachuug	Reference	2007	Channel	3m	5						
12	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2007	Outer	10m	1						
13	0	1128.66	0	0	0	0	0	0	0		Gachuug	Reference	2007	Outer	10m	2						
14	0	1838.304	0	0	0	0	0	0	0		Gachuug	Reference	2007	Outer	10m	3						
15	0	690.9934	0	0	0	0	0	0	0		Gachuug	Reference	2007	Outer	10m	4						
16	0	1249.616	0	0	0	2179.418	0	0	0		Gachuug	Reference	2007	Outer	10m	5						
17	0	0	0	0	0	0	5219.694	0	0		Gachuug	Reference	2007	Outer	3m	1						
18	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2007	Outer	3m	2						
19	0	0	0	0	0	0	1615.408	0	0		Gachuug	Reference	2007	Outer	3m	3						
20	0	0	0	0	0	0	949.0819	0	0		Gachuug	Reference	2007	Outer	3m	4						
21	0	0	0	0	0	0	1774.951	0	0		Gachuug	Reference	2007	Outer	3m	5						
22	0	0	0	0	0	0	0	30.97017	0		Gachuug	Reference	2009	Channel	10m	1						
23	0	40.93125	0	0	0	165.8322	0	0	0		Gachuug	Reference	2009	Channel	10m	2						
24	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	10m	3						
25	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	10m	4						
26	0	405.4788	0	3389.799	0	0	0	0	0		Gachuug	Reference	2009	Channel	10m	5						
27	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	3m	1						
28	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	3m	2						
29	13980.48	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	3m	3						
30	13980.48	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	3m	4						
31	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Channel	3m	5						
32	0	0	0	0	0	0	0	2329.665	0		Gachuug	Reference	2009	Outer	3m	1						
33	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Outer	3m	2						
34	0	0	0	0	0	0	0	0	0		Gachuug	Reference	2009	Outer	3m	3						

20. **Save <u>AND</u> Minimize** the Excel file.

21. **Open** the PRIMER-E Program.
    a. **Click** on the "***open file***" icon
    b. **Click** the arrow to open the drop down menu next to "***Files of type***:"
    c. **Set** this to Excel.



22. **Navigate** to your Excel file, **"Yap-Nimpal-MPA-Fish.xlsx",** and **click** open
23. **Click** on the dropdown menu that now appears under "***Excel worksheet***"
    a. **Select** the "Primer import" sheet we just made
    b. Make sure "***Sample data***" is **checked**
24. **Click** Next.

***Note**: We did not include a title in our Excel sheet*

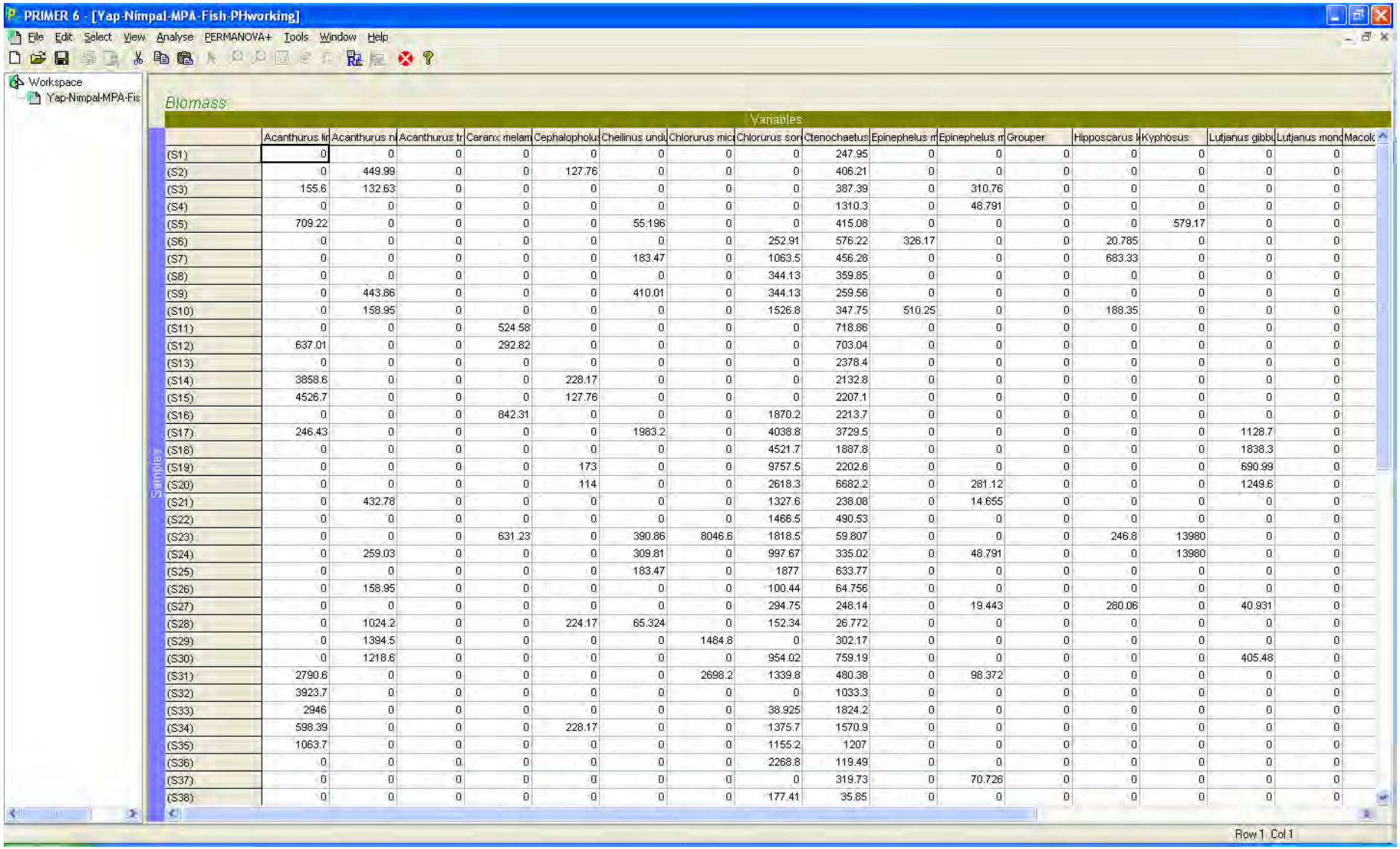
25. In the next dialog box
    a. **Uncheck** the green mark next to "***Title***".

***Note**: We also did not include Row labels in our Excel sheet,*

    b. Uncheck the green mark next to "***Row labels***".
    c. **Select** "***Samples as rows***" (*as our data are aligned in rows*)
26. **Select** "**Biomass**" for the type of data.

27. Click “Finish”.

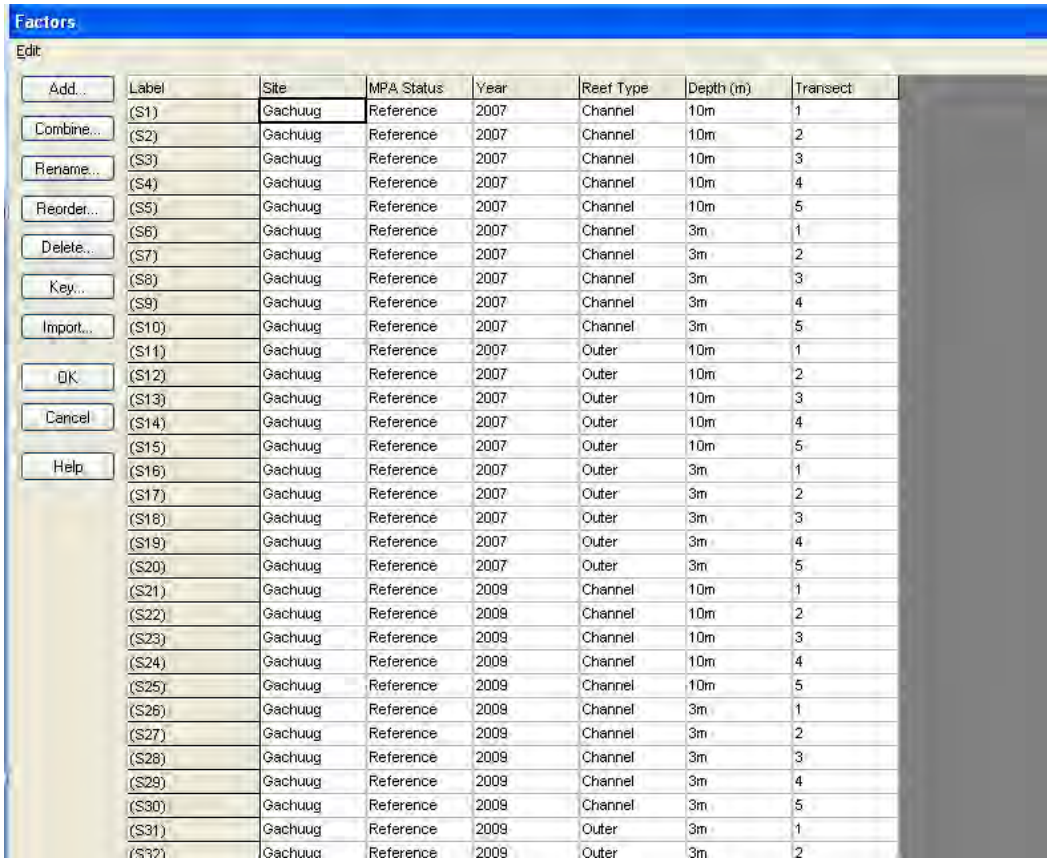
You should have now successfully imported your data into PRIMER, maximize the windows and confirm.



	Acanthurus lin	Acanthurus ni	Acanthurus tr	Caranx melam	Cephalopholus	Cheilinus undu	Chlorurus mic	Chlorurus sor	Ctenochaetus	Epinephelus m	Epinephelus m	Grouper	Hipposcarus l	Kyphosus	Lutjanus gibbu	Lutjanus mond	Macolo
(S1)	0	0	0	0	0	0	0	0	247.95	0	0	0	0	0	0	0	
(S2)	0	449.99	0	0	127.76	0	0	0	406.21	0	0	0	0	0	0	0	
(S3)	155.6	132.63	0	0	0	0	0	0	387.39	0	310.76	0	0	0	0	0	
(S4)	0	0	0	0	0	0	0	0	1310.3	0	48.791	0	0	0	0	0	
(S5)	709.22	0	0	0	0	55.196	0	0	415.08	0	0	0	0	579.17	0	0	
(S6)	0	0	0	0	0	0	0	252.91	576.22	326.17	0	0	20.785	0	0	0	
(S7)	0	0	0	0	0	183.47	0	1063.5	456.28	0	0	0	683.33	0	0	0	
(S8)	0	0	0	0	0	0	0	344.13	359.85	0	0	0	0	0	0	0	
(S9)	0	443.86	0	0	0	410.01	0	344.13	259.56	0	0	0	0	0	0	0	
(S10)	0	158.95	0	0	0	0	0	1526.8	347.75	510.25	0	0	188.35	0	0	0	
(S11)	0	0	0	524.58	0	0	0	0	718.86	0	0	0	0	0	0	0	
(S12)	637.01	0	0	292.82	0	0	0	0	703.04	0	0	0	0	0	0	0	
(S13)	0	0	0	0	0	0	0	0	2378.4	0	0	0	0	0	0	0	
(S14)	3858.6	0	0	0	228.17	0	0	0	2132.8	0	0	0	0	0	0	0	
(S15)	4526.7	0	0	0	127.76	0	0	0	2207.1	0	0	0	0	0	0	0	
(S16)	0	0	0	842.31	0	0	0	1870.2	2213.7	0	0	0	0	0	0	0	
(S17)	246.43	0	0	0	0	1983.2	0	4038.8	3729.5	0	0	0	0	0	1128.7	0	
(S18)	0	0	0	0	0	0	0	4521.7	1887.8	0	0	0	0	0	1838.3	0	
(S19)	0	0	0	0	173	0	0	9757.5	2202.6	0	0	0	0	0	690.99	0	
(S20)	0	0	0	0	114	0	0	2618.3	6682.2	0	281.12	0	0	0	1249.6	0	
(S21)	0	432.78	0	0	0	0	0	1327.6	238.08	0	14.655	0	0	0	0	0	
(S22)	0	0	0	0	0	0	0	1466.5	490.53	0	0	0	0	0	0	0	
(S23)	0	0	0	631.23	0	390.86	8046.6	1818.5	59.807	0	0	0	246.8	13980	0	0	
(S24)	0	259.03	0	0	0	309.81	0	997.67	335.02	0	48.791	0	0	13980	0	0	
(S25)	0	0	0	0	0	183.47	0	1877	633.77	0	0	0	0	0	0	0	
(S26)	0	158.95	0	0	0	0	0	100.44	64.756	0	0	0	0	0	0	0	
(S27)	0	0	0	0	0	0	0	294.75	248.14	0	19.443	0	280.06	0	40.931	0	
(S28)	0	1024.2	0	0	224.17	65.324	0	152.34	26.772	0	0	0	0	0	0	0	
(S29)	0	1394.5	0	0	0	0	1484.8	0	302.17	0	0	0	0	0	0	0	
(S30)	0	1218.6	0	0	0	0	0	954.02	759.19	0	0	0	0	0	405.48	0	
(S31)	2790.6	0	0	0	0	0	2698.2	1339.8	480.38	0	98.372	0	0	0	0	0	
(S32)	3923.7	0	0	0	0	0	0	0	1033.3	0	0	0	0	0	0	0	
(S33)	2946	0	0	0	0	0	0	38.925	1824.2	0	0	0	0	0	0	0	
(S34)	598.39	0	0	0	228.17	0	0	1375.7	1570.9	0	0	0	0	0	0	0	
(S35)	1063.7	0	0	0	0	0	0	1155.2	1207	0	0	0	0	0	0	0	
(S36)	0	0	0	0	0	0	0	2268.8	119.49	0	0	0	0	0	0	0	
(S37)	0	0	0	0	0	0	0	0	319.73	0	70.726	0	0	0	0	0	
(S38)	0	0	0	0	0	0	0	177.41	35.85	0	0	0	0	0	0	0	

We will now view our factors that we wish to use in our analysis.

28. In PRIMER, **Click** on "***Edit***" menu and scroll down to and select "***Factors***". Confirm with screen shot below. These are all of the metadata columns in our excel sheet that we moved to the right of our data block.



Label	Site	MPA Status	Year	Reef Type	Depth (m)	Transect
(S1)	Gachuug	Reference	2007	Channel	10m	1
(S2)	Gachuug	Reference	2007	Channel	10m	2
(S3)	Gachuug	Reference	2007	Channel	10m	3
(S4)	Gachuug	Reference	2007	Channel	10m	4
(S5)	Gachuug	Reference	2007	Channel	10m	5
(S6)	Gachuug	Reference	2007	Channel	3m	1
(S7)	Gachuug	Reference	2007	Channel	3m	2
(S8)	Gachuug	Reference	2007	Channel	3m	3
(S9)	Gachuug	Reference	2007	Channel	3m	4
(S10)	Gachuug	Reference	2007	Channel	3m	5
(S11)	Gachuug	Reference	2007	Outer	10m	1
(S12)	Gachuug	Reference	2007	Outer	10m	2
(S13)	Gachuug	Reference	2007	Outer	10m	3
(S14)	Gachuug	Reference	2007	Outer	10m	4
(S15)	Gachuug	Reference	2007	Outer	10m	5
(S16)	Gachuug	Reference	2007	Outer	3m	1
(S17)	Gachuug	Reference	2007	Outer	3m	2
(S18)	Gachuug	Reference	2007	Outer	3m	3
(S19)	Gachuug	Reference	2007	Outer	3m	4
(S20)	Gachuug	Reference	2007	Outer	3m	5
(S21)	Gachuug	Reference	2009	Channel	10m	1
(S22)	Gachuug	Reference	2009	Channel	10m	2
(S23)	Gachuug	Reference	2009	Channel	10m	3
(S24)	Gachuug	Reference	2009	Channel	10m	4
(S25)	Gachuug	Reference	2009	Channel	10m	5
(S26)	Gachuug	Reference	2009	Channel	3m	1
(S27)	Gachuug	Reference	2009	Channel	3m	2
(S28)	Gachuug	Reference	2009	Channel	3m	3
(S29)	Gachuug	Reference	2009	Channel	3m	4
(S30)	Gachuug	Reference	2009	Channel	3m	5
(S31)	Gachuug	Reference	2009	Outer	3m	1
(S32)	Gachuug	Reference	2009	Outer	3m	2

29. Click OK

Now we are set for our analyses with PRIMER.

30. Save your workspace as a new PRIMER file called "***Yap-multivariate-fish-exercise***".

First, a note about PRIMER: this is a very powerful and user-friendly data visualization and analyses package. In this exercise we will cover some of the basic features. Each user at this workshop was provided a user manual and example guidebook that accompanies the software. As your capacity develops and your datasets emerge and change, you can refer to the manual for more examples and suggestions. Here, we will conduct some of the most basic procedures in PRIMER that shows how easy and powerful a multivariate approach to data analysis can be. It should be understood that less care is given to explaining the mathematical calculations that accompany these procedures; rather we focus mostly on visualizing and testing patterns and leave it to the user manual to describe the math behind each operation.

We will first take a multivariate look at the differences in fish assemblages for shallow coral assemblages inside the Nimpal no-take preserve, and the Gachuug reference location.

31. **Select** the samples that we wish to compare.
32. From the PRIMER ***main menu***,
    a. **Click** on "***edit***" and **scroll down** to "***factors***".

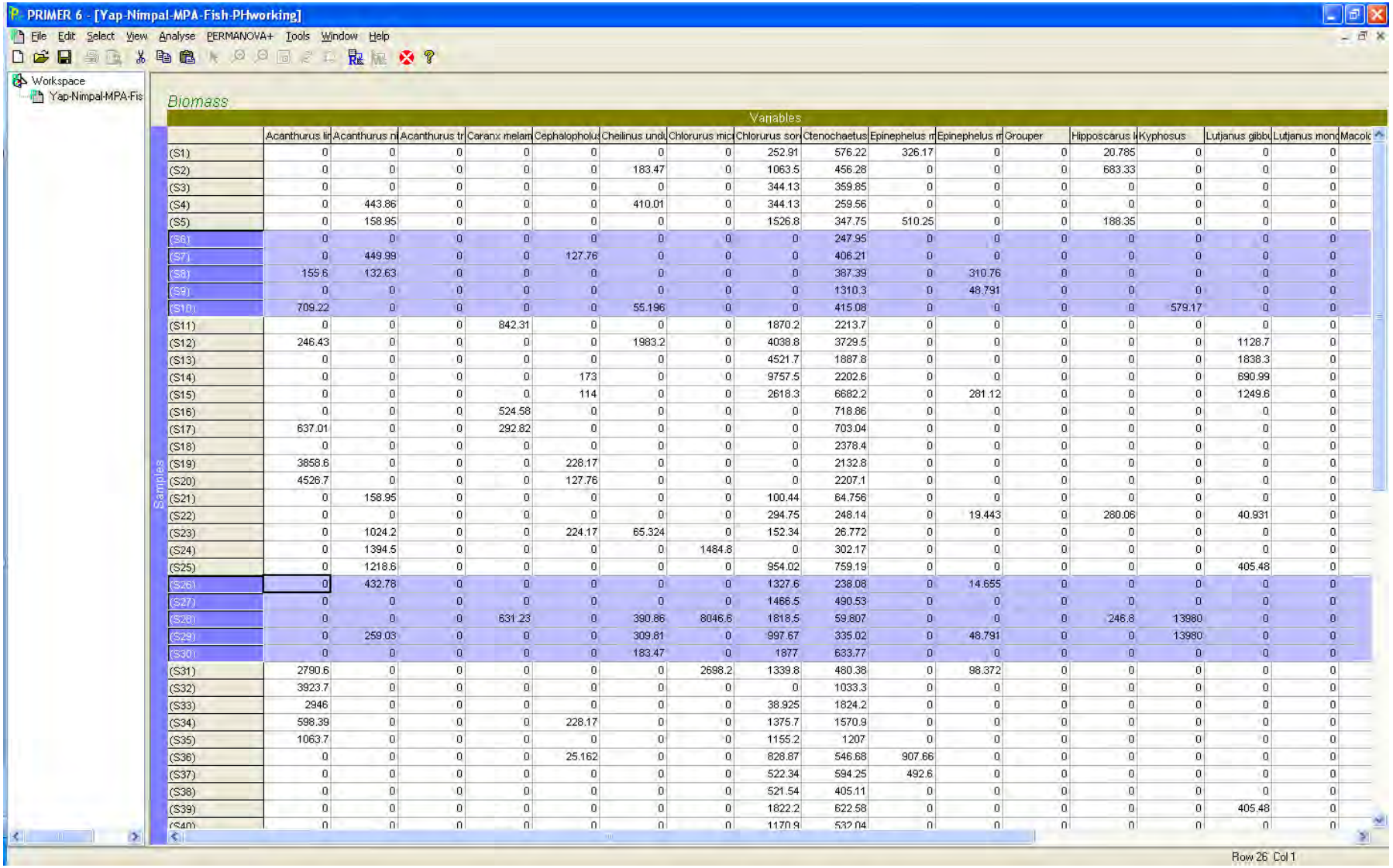
Note*: Get some scratch paper and a pencil ready.*

27. **Maximize** the "***Factors***" dialog box to the entire screen

We will first look at the differences in fish assemblages between the Nimpal conservation area and the Gachuug reference site for the "Channel" reef type, and only at "3m". We want to record all "Labels", or sample ID's, that pertain to our analyses so we can select them from the main screen.

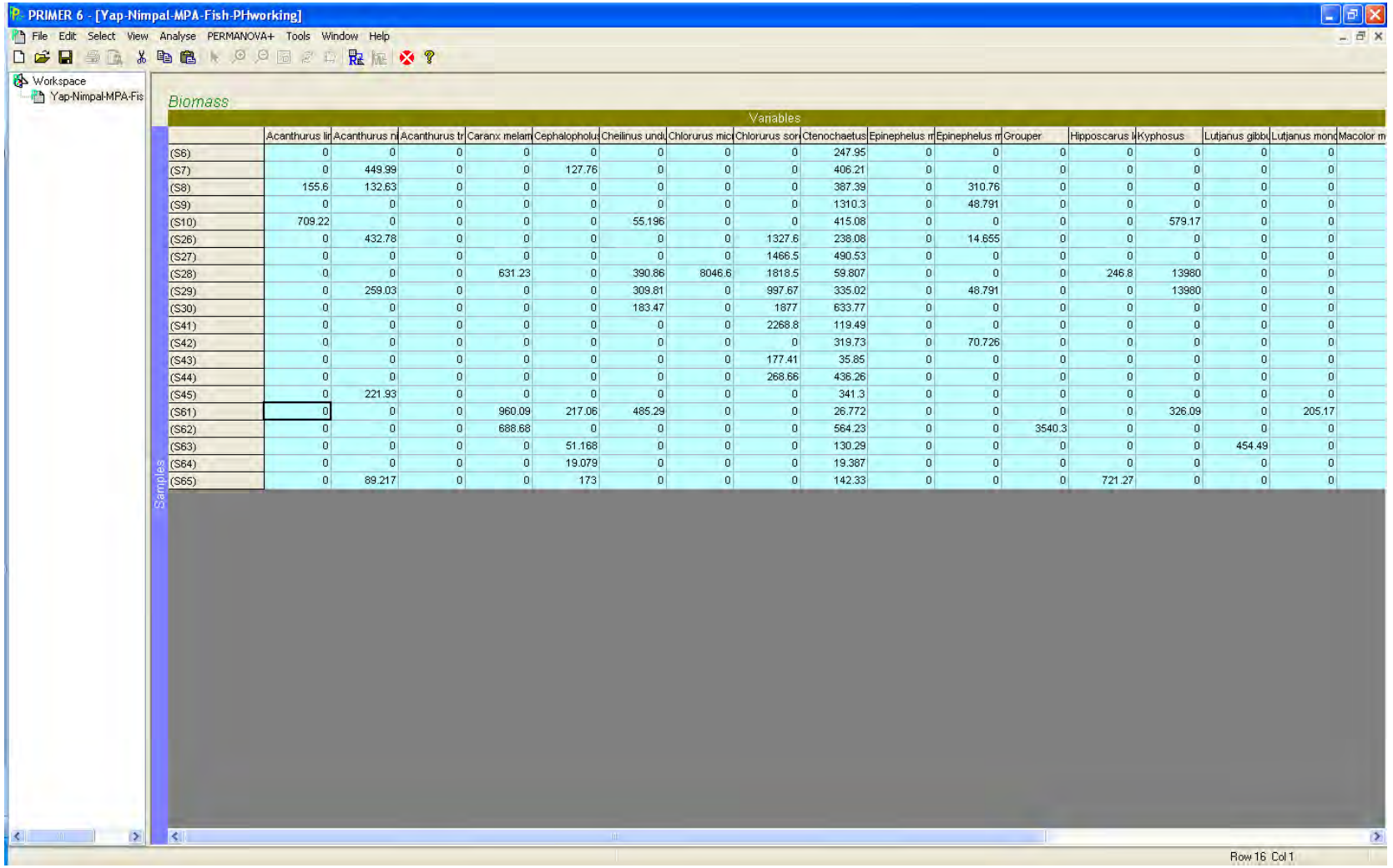
Important: Confirm *on your own that for the analyses defined above we wish to examine sites (or Labels) (S6-10), (S26-30), (S41-45), and (S61-65). These correspond to both Nimpal and Gachuug samples, channel reef types only, and 3m depth only.*

28. **Close** the ***Factors*** window.
29. On your main data sheet **highlight** the rows that pertain to our ***desired analysis*** by **left clicking** on each.
30. Confirm below.



33. Once you have selected the appropriate samples, **navigate** to the **"Select"** main menu and scroll down to **"Highlighted"**.

a. Confirm below



	Acanthurus lir	Acanthurus ni	Acanthurus tr	Caranx melam	Cephalopholus	Cheilinus undu	Chlorurus mic	Chlorurus sor	Ctenochaetus	Epinephelus m	Epinephelus m	Grouper	Hipposcarus l	Kyphosus	Lutjanus gibb	Lutjanus mon	Macolor m
(S6)	0	0	0	0	0	0	0	0	247.95	0	0	0	0	0	0	0	
(S7)	0	449.99	0	0	127.76	0	0	0	406.21	0	0	0	0	0	0	0	
(S8)	155.6	132.63	0	0	0	0	0	0	387.39	0	310.76	0	0	0	0	0	
(S9)	0	0	0	0	0	0	0	0	1310.3	0	48.791	0	0	0	0	0	
(S10)	709.22	0	0	0	0	55.196	0	0	415.08	0	0	0	0	579.17	0	0	
(S26)	0	432.78	0	0	0	0	0	1327.6	238.08	0	14.655	0	0	0	0	0	
(S27)	0	0	0	0	0	0	0	1466.5	490.53	0	0	0	0	0	0	0	
(S28)	0	0	0	631.23	0	390.86	8046.6	1818.5	59.807	0	0	0	246.8	13980	0	0	
(S29)	0	259.03	0	0	0	309.81	0	997.67	335.02	0	48.791	0	0	13980	0	0	
(S30)	0	0	0	0	0	183.47	0	1877	633.77	0	0	0	0	0	0	0	
(S41)	0	0	0	0	0	0	0	2268.8	119.49	0	0	0	0	0	0	0	
(S42)	0	0	0	0	0	0	0	0	319.73	0	70.726	0	0	0	0	0	
(S43)	0	0	0	0	0	0	0	177.41	35.85	0	0	0	0	0	0	0	
(S44)	0	0	0	0	0	0	0	268.66	436.26	0	0	0	0	0	0	0	
(S45)	0	221.93	0	0	0	0	0	0	341.3	0	0	0	0	0	0	0	
(S61)	0	0	0	960.09	217.06	485.29	0	0	26.772	0	0	0	0	326.09	0	205.17	
(S62)	0	0	0	688.68	0	0	0	0	564.23	0	0	3540.3	0	0	0	0	
(S63)	0	0	0	0	51.168	0	0	0	130.29	0	0	0	0	0	454.49	0	
(S64)	0	0	0	0	19.079	0	0	0	19.387	0	0	0	0	0	0	0	
(S65)	0	89.217	0	0	173	0	0	0	142.33	0	0	0	721.27	0	0	0	

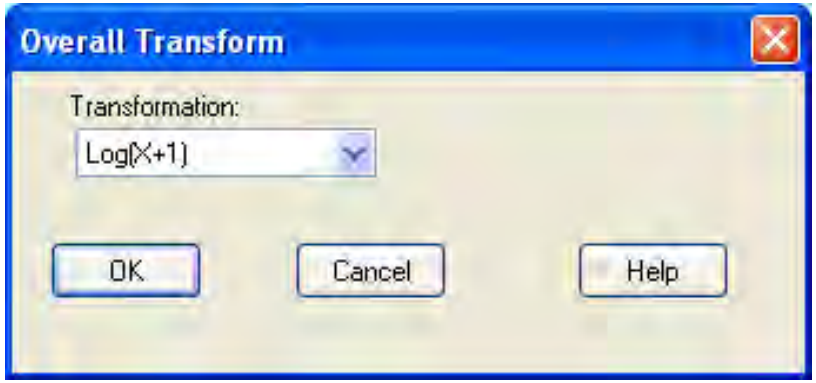
You will notice the color of the cells changes to blue to indicate that selective conditions for samples are in place. (Note: each row represents one individual transect from which observations were made.)

Next, we are ready to conduct a basic data transformation, a log transformation, so that our analyses takes into account the dominant and rare species of fish recorded in a more realistic manner as they existed at the study sites. Without the transformation, dominant fish such as 'Ctenochaetus striatus' would have a dominant influence on the multivariate assessment. While this species is commonplace to most reefs on Yap, our goal is to take the entire assemblage into account. You can confer with the user manual to

better understand the concepts behind data transformations. Also, the topic of transforming data has been heavily documented in books and scientific articles. The transformation one chooses typically depends upon the type of data that was collected. Count data typically utilize a different transformation as compared with biomass and percent coverage data. The transformation selected for use here is widely accepted and commonly employed for biomass and abundance data.

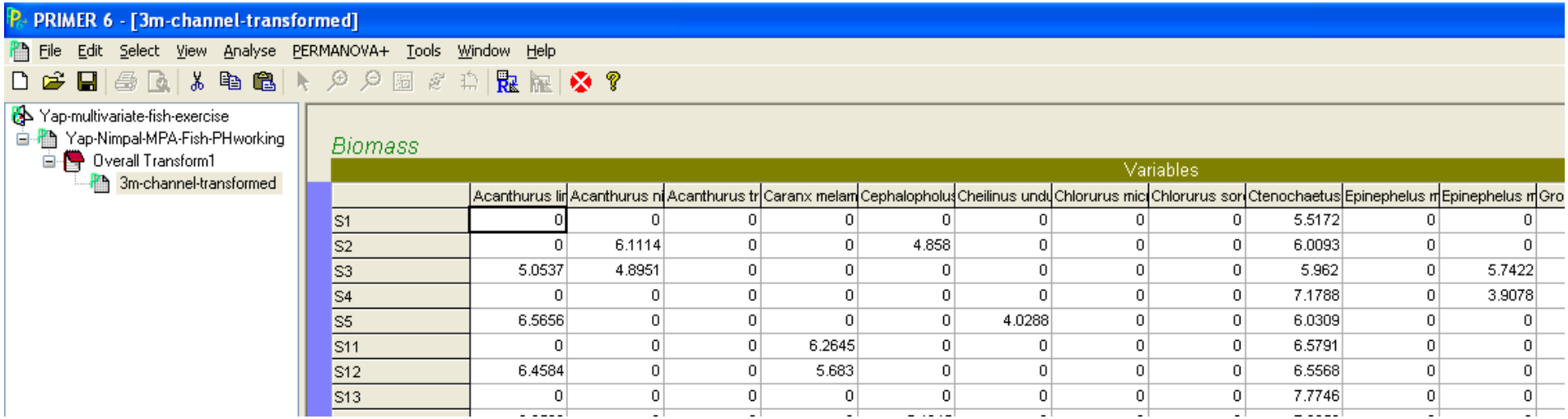
34. Goto the "***Analyze***" main menu,

1. **Scroll down to** "***pre-treatment***"
2. **Select** "***Transform overall***".
3. **Select *Log (X+1)*** from the drop down menu
4. **Click** OK.
5. Confirm Below



You have now created a new species by site datasheet, you can see on the left that the current name is "Data1".

35. Rename this to "***3m-channel-transformed***".

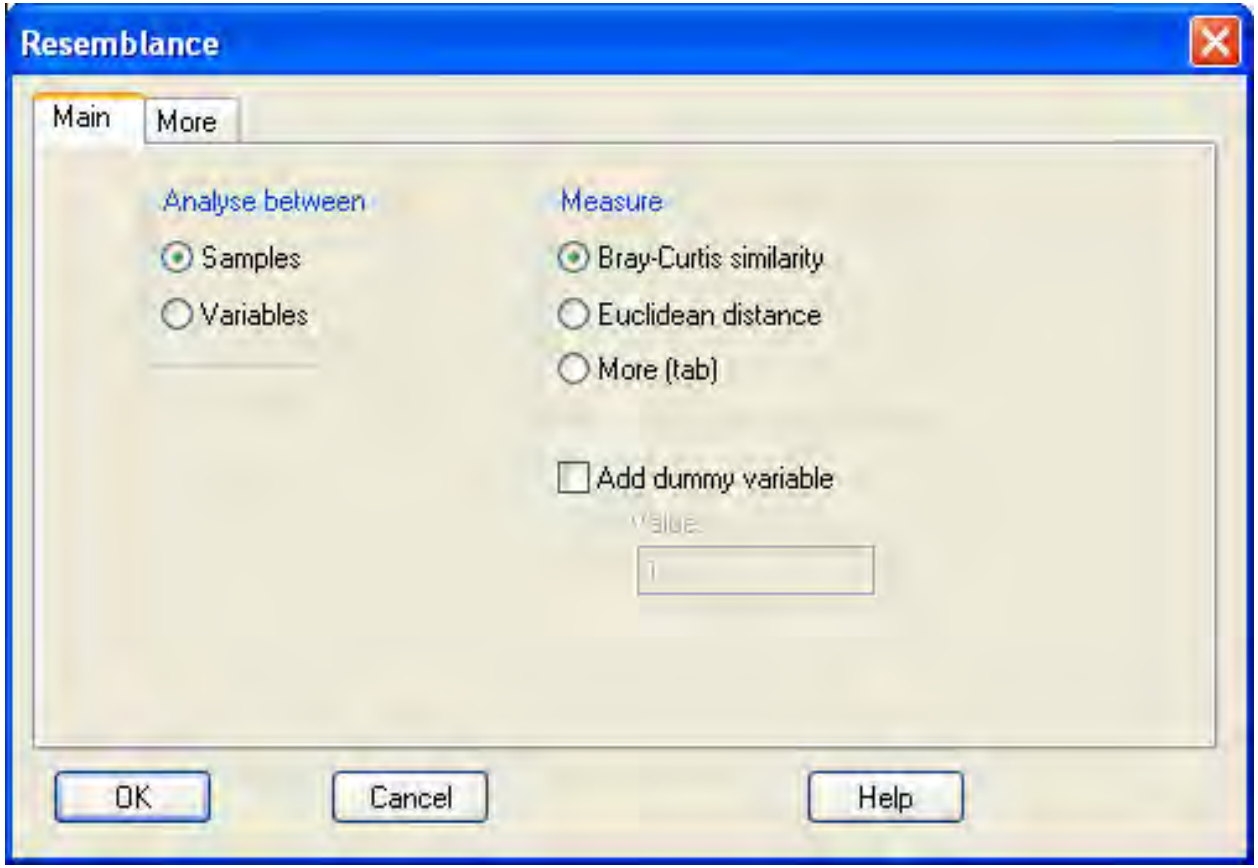


We will now proceed and create a similarity matrix. A similarity matrix compares each individual sample (our 'sample' unit is one individual transect remember) to others based upon the differing biomass of fish in each species category. Again there are many mathematical formulas that researchers have derived to do this, we'll use a very common one for ecological studies called a "Bray-Curtis" similarity measure. Here is what it looks like:

$$D(y1, y2) = \Sigma |y1j - y2j| / \Sigma (y1j + y2j)$$

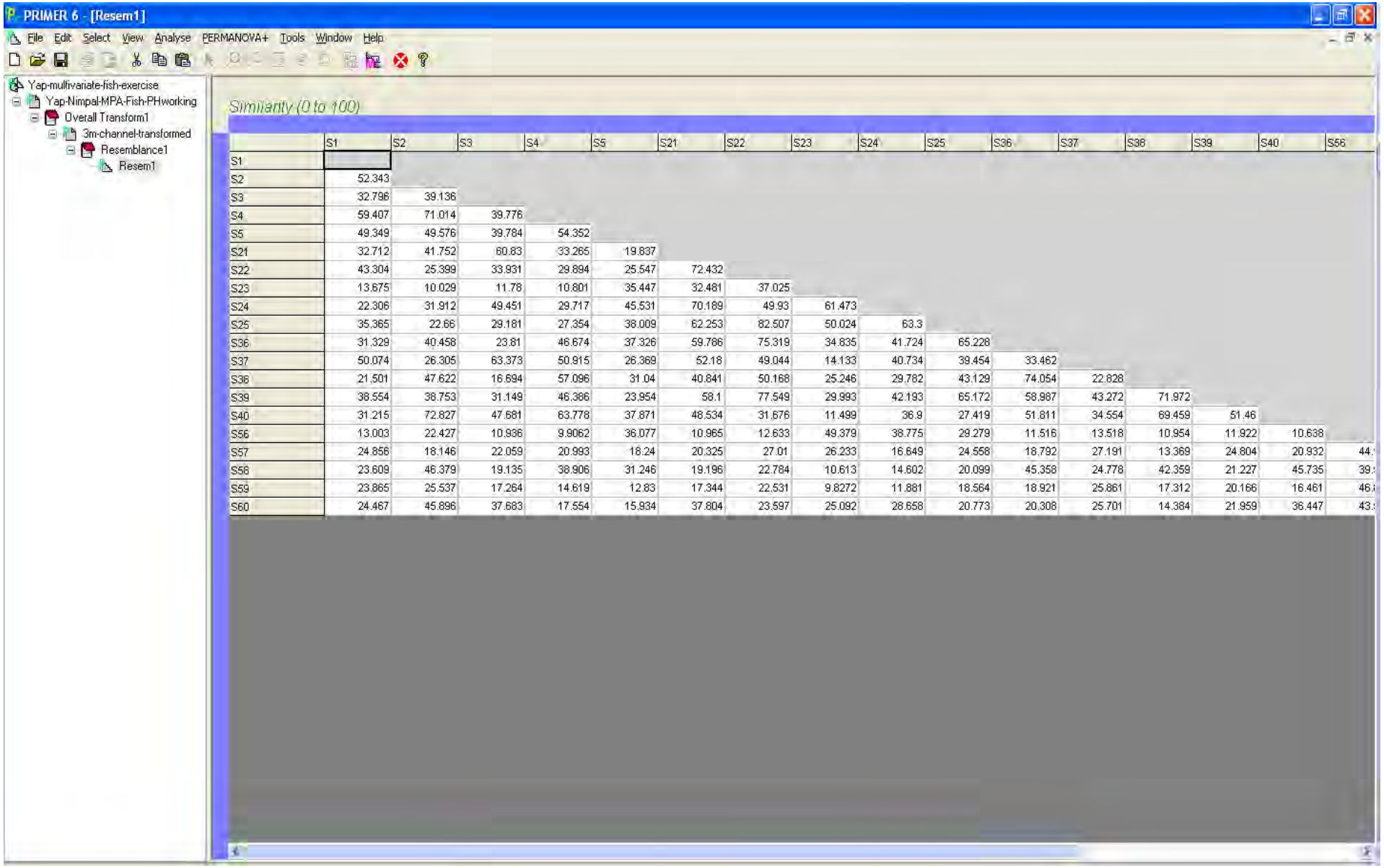
*D is the Bray-Curtis distance between two samples (or transects in our case). Σ represents the summation for all fish species and y1j and y2j represent fish biomass from two different transects. It is simple to understand that the ecological distance is calculated by dividing the **difference** between fish biomass by the **sum**, for **two consecutive transects**. This is done for all species and all transects by the computer, and we end up with desirable measure of distance between each pair of transects. In other words, the distance tells us how similar two transects were, or were not.*

36. Go to the "***Analyze***" menu
   a. Select "***Resemblance***".



Note: Make sure the analysis is between "***Samples***" and were using the "***Bray-Curtis***" similarity.

37. Click OK.



	S1	S2	S3	S4	S5	S21	S22	S23	S24	S25	S36	S37	S38	S39	S40	S56
S1																
S2	52.343															
S3	32.796	39.136														
S4	59.407	71.014	39.776													
S5	49.349	49.576	39.784	54.352												
S21	32.712	41.752	60.83	33.265	19.837											
S22	43.304	25.399	33.931	29.894	25.547	72.432										
S23	13.675	10.029	11.78	10.801	35.447	32.481	37.025									
S24	22.306	31.912	49.451	29.717	45.531	70.189	49.93	61.473								
S25	35.365	22.66	29.181	27.354	38.009	62.253	82.507	50.024	63.3							
S36	31.329	40.458	23.81	46.674	37.326	59.786	75.319	34.835	41.724	65.228						
S37	50.074	26.305	63.373	50.915	26.369	52.18	49.044	14.133	40.734	39.454	33.462					
S38	21.501	47.622	16.694	57.096	31.04	40.841	50.168	25.246	29.782	43.129	74.054	22.828				
S39	38.554	38.753	31.149	46.386	23.954	58.1	77.549	29.993	42.193	65.172	58.987	43.272	71.972			
S40	31.215	72.827	47.681	63.778	37.871	48.534	31.676	11.499	36.9	27.419	51.811	34.554	69.459	51.46		
S56	13.003	22.427	10.936	9.9062	36.077	10.965	12.633	49.379	38.775	29.279	11.516	13.518	10.954	11.922	10.638	
S57	24.856	18.146	22.059	20.993	18.24	20.325	27.01	26.233	16.649	24.558	18.792	27.191	13.369	24.804	20.932	44.
S58	23.609	46.379	19.135	38.906	31.246	19.196	22.784	10.613	14.602	20.099	45.358	24.778	42.359	21.227	45.735	39.
S59	23.865	25.537	17.264	14.619	12.83	17.344	22.531	9.8272	11.881	18.564	18.921	25.861	17.312	20.166	16.461	46.
S60	24.467	45.896	37.683	17.554	15.934	37.804	23.597	25.092	28.658	20.773	20.308	25.701	14.384	21.959	36.447	43.

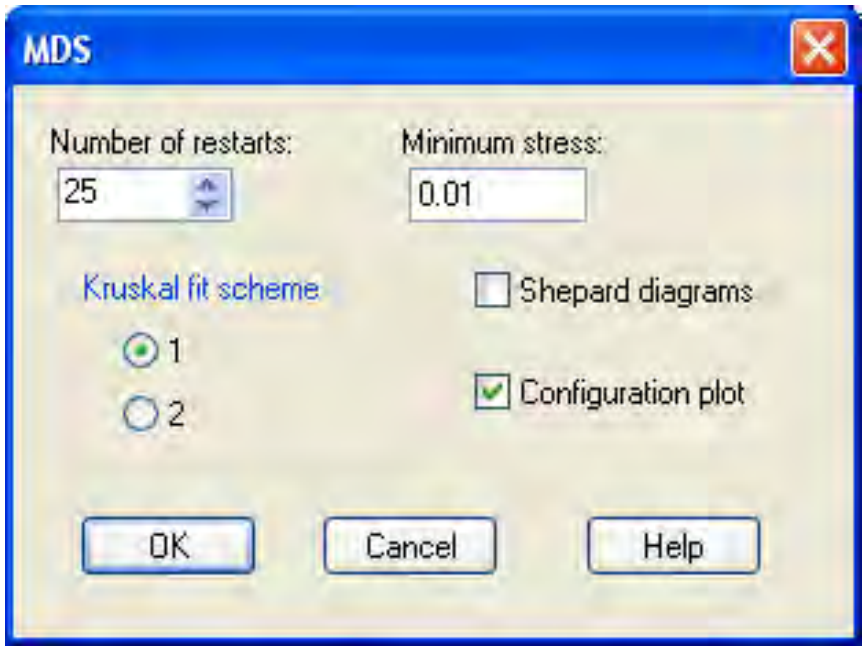
Now we have a "data matrix" that compares every possible combination of transects, and provides a distance measure of ecological similarity for each comparison.

Note that we could have used many different similarity indices besides the Bray-Curtis, you can learn about these and when they are appropriate from your user manual.

From here we want to visualize our findings. PRIMER, again, has many options for the user to consider. We will use the most common visualization method called "multi-dimensional scaling". Through this process the distances we calculate between each pair of sites are ranked from lowest to highest using an ordinal scale, and the resultant plot highlights these relationships, specifically, how the pairwise relationships all fit into a bigger picture, called "multi-dimensional" space. The computer then reduces the dimension of the resultant plot down to two or three, while preserving as much of the structure in the data as possible. It is best understood through an example, and the math behind this can be found in the user manual.

38. Go to the "***Analyze***" menu.
(Notice the options have changed, items that were previously available are no longer. This is because we are working with an active 'resemblance matrix' as opposed to a 'species by site' dataset.)

a. **Select** MDS.

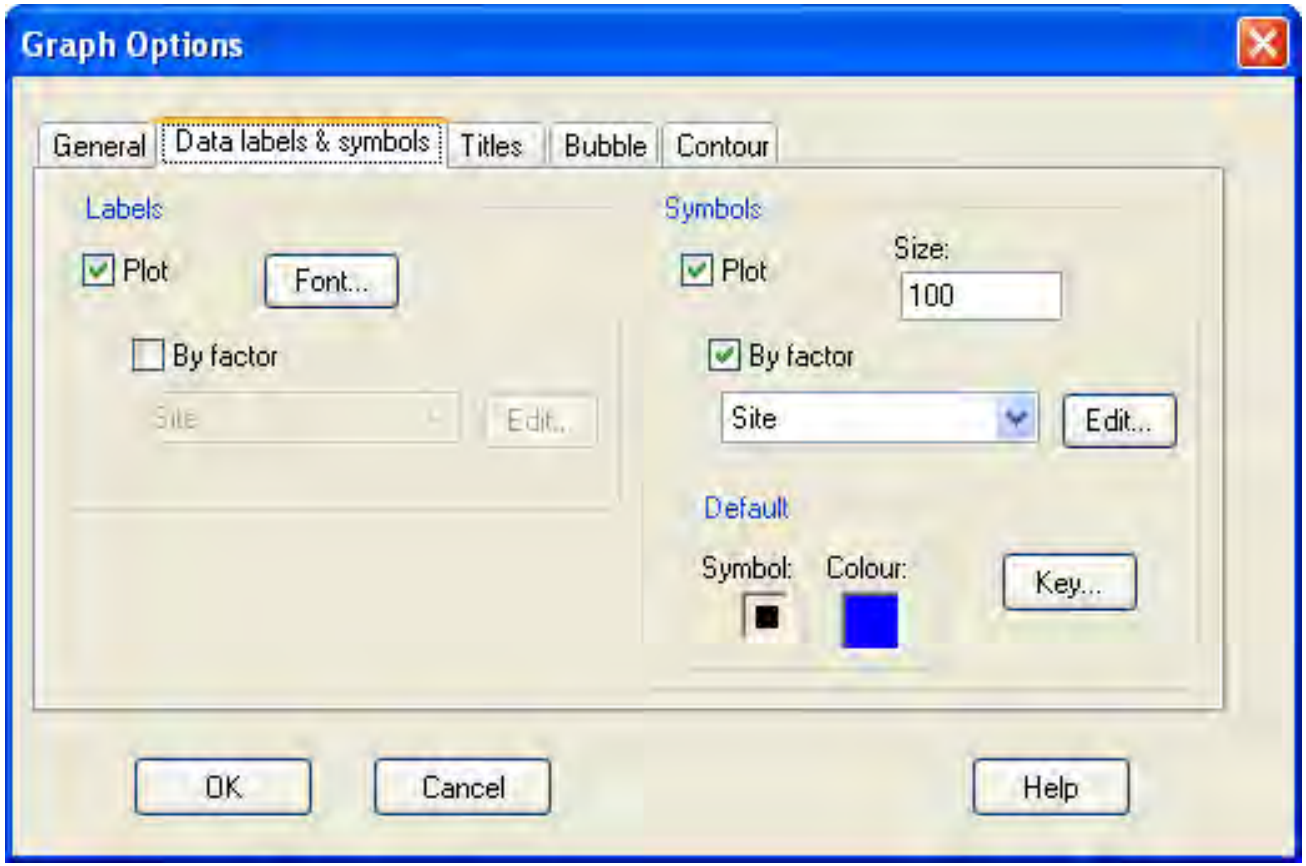


b. **Keep** the ***default settings*** for our options
c. **Click** OK.

After a bit of processing time, PRIMER produces a 2-dimensional and 3 dimensional plot called “Graph1 and Graph2”. Let’s just focus on the first, 2-dimensional plot. We will change the look of this plot to better understand the findings.

39. Under the “***Graph***” menu

- **a. Select** “***Data labels & symbols***”.



40. For “***Labels***”

- **a. Check** the “***By factor***” box
- **b.** From the drop down menu **select** “***Year***”

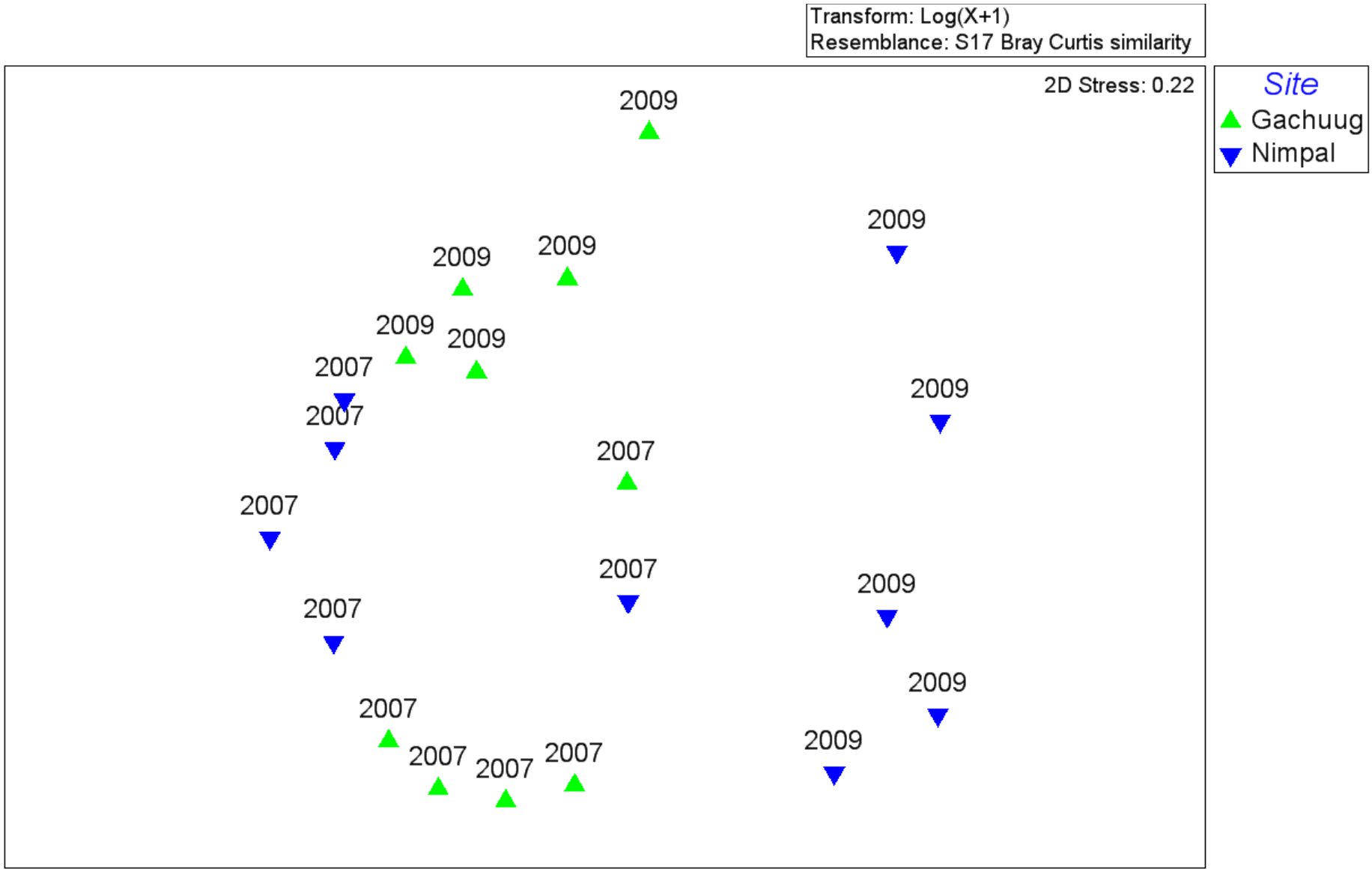
41. For “Symbols”

- **a. Check** the “***By factor***” box
- **b.** From the drop down menu **select** “***Site***”

42. Click ok

43. Confirm

*(**Note:** Your graph may be rotated differently, however the spatial distances between sites should be the same.)*



Take a moment to reflect what we learn from this graph. First, in the upper right corner we see "2D Stress: 0.22". This tells us how successful our MDS plot has maintained the actual ecological distances between each transect, while transforming the output into only 2 dimensions. The user manual provides references to research that suggest that values of 0.25 or below are typically considered sufficient and reliable. So, we have successfully portrayed our data into 2-dimensions, and don't need to look at the 3-dimension graph, unless your interested.

Most notably, however, the graph tells us that for inner channel sites, 3m fish biomass data have changed for the Nimpal site between 2007 and 2009. This is not true for all transects, but for many the trend holds. However for Gachuug, the fish biomass did not change. So, we have indication that change occurred only at Nimpal, the MPA, but we need to understand what the 'change' is.

Next we will calculate the contribution of each species of fish to our detected trends. PRIMER has a built in analyses that calculates the relative contributions of each species in determining the trends that the graph show.

44. Go back to the "***3m-channel-transformed***" data sheet.

We need to make further selections from our data. What we want to know is how and why the fish biomass are different between these reefs in 2009 only, because in 2007 they were still similar. Basically, we'd like to know what change occurred.

45. In the "***Edit***" menu, then **select** "***Factors***".

Notice only our subset of sites appears.

For our next examination we wish to look at only 2009 data, corresponding to samples (S26-30) and (S61-65). Note those sample labels on your scratch paper and close the factors box.

46. On the main datasheet highlight only the new ***samples*** noted above,
47. Go to the "***Select***" menu
   a. Select "***highlighted"***

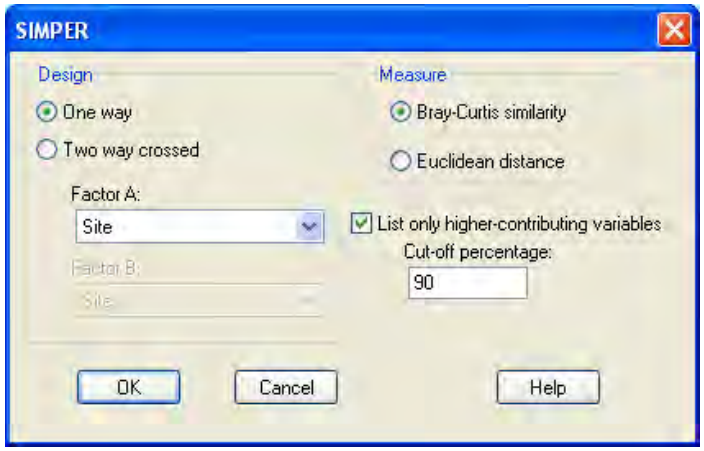
48. Confirm your datasheet below.

(Notice only 10 samples remain, these correspond to 10 transects surveyed, 5 inside of the MPA at a 3m depth in 2009, and 5 outside.)

Biomass

	Variables																
	Acanthurus lir	Acanthurus ni	Acanthurus tr	Caranx melam	Cephalopholus	Cheilinus undu	Chlorurus mici	Chlorurus sor	Ctenochaetus	Epinephelus m	Epinephelus m	Grouper	Hipposcarus l	Kyphosus	Lutjanus gibbu	Lutjanus mond	Macolor m
(S26)	0	432.78	0	0	0	0	0	1327.6	238.08	0	14.655	0	0	0	0	0	
(S27)	0	0	0	0	0	0	0	1466.5	490.53	0	0	0	0	0	0	0	
(S28)	0	0	0	631.23	0	390.86	8046.6	1818.5	59.807	0	0	0	246.8	13980	0	0	
(S29)	0	259.03	0	0	0	309.81	0	997.67	335.02	0	48.791	0	0	13980	0	0	
(S30)	0	0	0	0	0	183.47	0	1877	633.77	0	0	0	0	0	0	0	
(S61)	0	0	0	960.09	217.06	485.29	0	0	26.772	0	0	0	0	326.09	0	205.17	
(S62)	0	0	0	688.68	0	0	0	0	564.23	0	0	3540.3	0	0	0	0	
(S63)	0	0	0	0	51.168	0	0	0	130.29	0	0	0	0	0	454.49	0	
(S64)	0	0	0	0	19.079	0	0	0	19.387	0	0	0	0	0	0	0	
(S65)	0	89.217	0	0	173	0	0	0	142.33	0	0	0	721.27	0	0	0	

49. Go to the "***Analyze***" menu and **select** "***SIMPER***" *(which is short for analyses of similarities).*



50. Under "Factor A:"

- **a. Select** "***Site***"

So we can determine differences can leave the default settings that match our MDS plot generation

51. Click OK.

52. Confirm

(Note: Scroll down the text output sheet so we can see the comparison between the two sites. The relevant section was manually highlighted in blue for identification)

Groups Gachuug & Nimpal
Average dissimilarity = 77.63

Species	Group Gachuug Av.Abund	Group Nimpal Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Chlorurus sordidus	7.29	0.00	14.03	3.08	18.07	18.07
Scarus sp.	0.00	6.80	13.22	2.73	17.02	35.10
Cephalopholus argus	0.00	3.50	6.62	1.72	8.53	43.62
Kyphosus	3.82	1.16	6.12	0.92	7.89	51.51
Cheilinus undulatus	3.39	1.24	5.67	1.09	7.30	58.81
Caranx melampygus	1.29	2.68	4.85	0.85	6.25	65.07
Acanthurus nigricauda	2.33	0.90	4.64	0.86	5.97	71.04
Hipposcarus longiceps	1.10	1.32	3.33	0.64	4.29	75.33
Ctenochaetus striatus	5.61	4.50	3.22	1.07	4.14	79.47
Grouper	0.00	1.63	2.83	0.48	3.64	83.11
Plectorhinchus lineatus	0.00	1.43	2.62	0.48	3.38	86.49
Chlorurus microrhinos	1.80	0.00	2.38	0.49	3.07	89.56
Epinephelus merra	1.33	0.00	2.34	0.78	3.02	92.58

From this table three columns are most informative. The first column has the average biomass from Gachuug (the reference site) for each fish species. The second from Nimpal. For now, we can disregard the next two columns and focus upon the % contribution. We are most interested in what species contributed to the majority of the difference found in our MDS plot. Notice the first four fish cumulatively accounted for > 50% of the variance (the last column tells us the cumulative variance accounted for). So we should logically focus upon these four species. The most notable difference are a shift in parrotfish from Chlorurus sordidus, very common at the reference site, to 'other Scarids' (including Hipposcarus longiceps, Scarus forsteni, S. frenatus, and others). Also, there has been an increase in the grouper (Cephalopholus argus).

Now we have a good idea of where change occurred, the magnitude of change, and what 'change' consisted of. This is very powerful to aid our understanding.

Let's continue to look at other reef types and depths.

53. Go back to the first, main data sheet under the "***Yap-multivariate-fish-exercise***".
54. From the ***select*** menu, **select** "***All***".

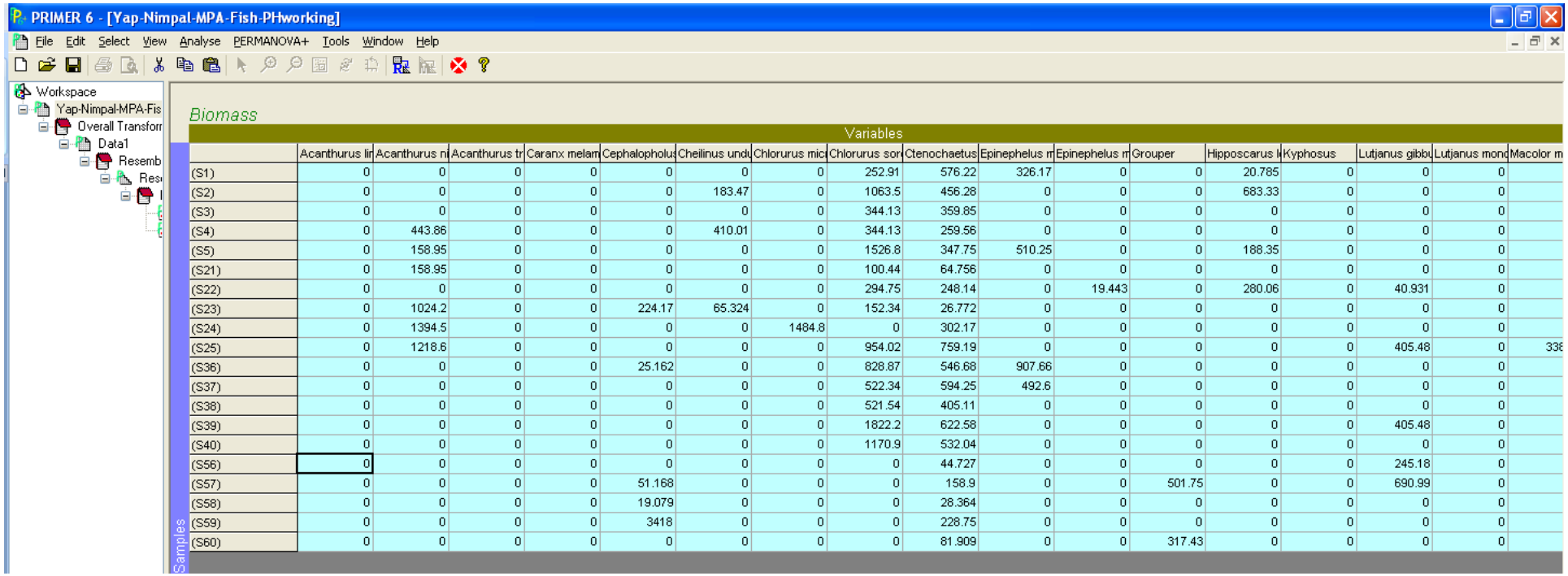
55. Go back to the "***Edit***" menu and **select** "Factors".

Let's look at the same channel reefs, this time at the 10m depth.

56. On your scratch paper **record** the ***relevant sites*** we want to highlight **(*S1-5*)**, **(*S21-25*)**, **(*S36-40*)**, and **(*S56-60*)**.

(*Note:* You can deselect the undesired samples by clicking on them, and select the new samples noted above)

57. From the "***Select***" menu **choose** "***Highlighted***".
58. Confirm.

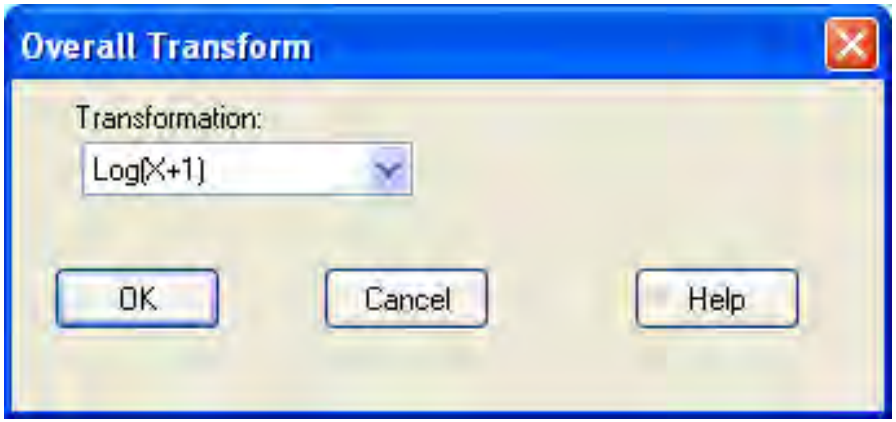


	Acanthurus lin	Acanthurus n	Acanthurus tr	Caranx melam	Cephalopholu	Cheilinus undu	Chlorurus mic	Chlorurus sor	Ctenochaetus	Epinephelus m	Epinephelus m	Grouper	Hipposcarus h	Kyphosus	Lutjanus gibbu	Lutjanus mond	Macolor m
(S1)	0	0	0	0	0	0	0	252.91	576.22	326.17	0	0	20.785	0	0	0	
(S2)	0	0	0	0	0	183.47	0	1063.5	456.28	0	0	0	683.33	0	0	0	
(S3)	0	0	0	0	0	0	0	344.13	359.85	0	0	0	0	0	0	0	
(S4)	0	443.86	0	0	0	410.01	0	344.13	259.56	0	0	0	0	0	0	0	
(S5)	0	158.95	0	0	0	0	0	1526.8	347.75	510.25	0	0	188.35	0	0	0	
(S21)	0	158.95	0	0	0	0	0	100.44	64.756	0	0	0	0	0	0	0	
(S22)	0	0	0	0	0	0	0	294.75	248.14	0	19.443	0	280.06	0	40.931	0	
(S23)	0	1024.2	0	0	224.17	65.324	0	152.34	26.772	0	0	0	0	0	0	0	
(S24)	0	1394.5	0	0	0	0	1484.8	0	302.17	0	0	0	0	0	0	0	
(S25)	0	1218.6	0	0	0	0	0	954.02	759.19	0	0	0	0	0	405.48	0	338
(S36)	0	0	0	0	25.162	0	0	828.87	546.68	907.66	0	0	0	0	0	0	
(S37)	0	0	0	0	0	0	0	522.34	594.25	492.6	0	0	0	0	0	0	
(S38)	0	0	0	0	0	0	0	521.54	405.11	0	0	0	0	0	0	0	
(S39)	0	0	0	0	0	0	0	1822.2	622.58	0	0	0	0	0	405.48	0	
(S40)	0	0	0	0	0	0	0	1170.9	532.04	0	0	0	0	0	0	0	
(S56)	0	0	0	0	0	0	0	0	44.727	0	0	0	0	0	245.18	0	
(S57)	0	0	0	0	51.168	0	0	0	158.9	0	0	501.75	0	0	690.99	0	
(S58)	0	0	0	0	19.079	0	0	0	28.364	0	0	0	0	0	0	0	
(S59)	0	0	0	0	3418	0	0	0	228.75	0	0	0	0	0	0	0	
(S60)	0	0	0	0	0	0	0	0	81.909	0	0	317.43	0	0	0	0	

We will follow the exact same steps as before.

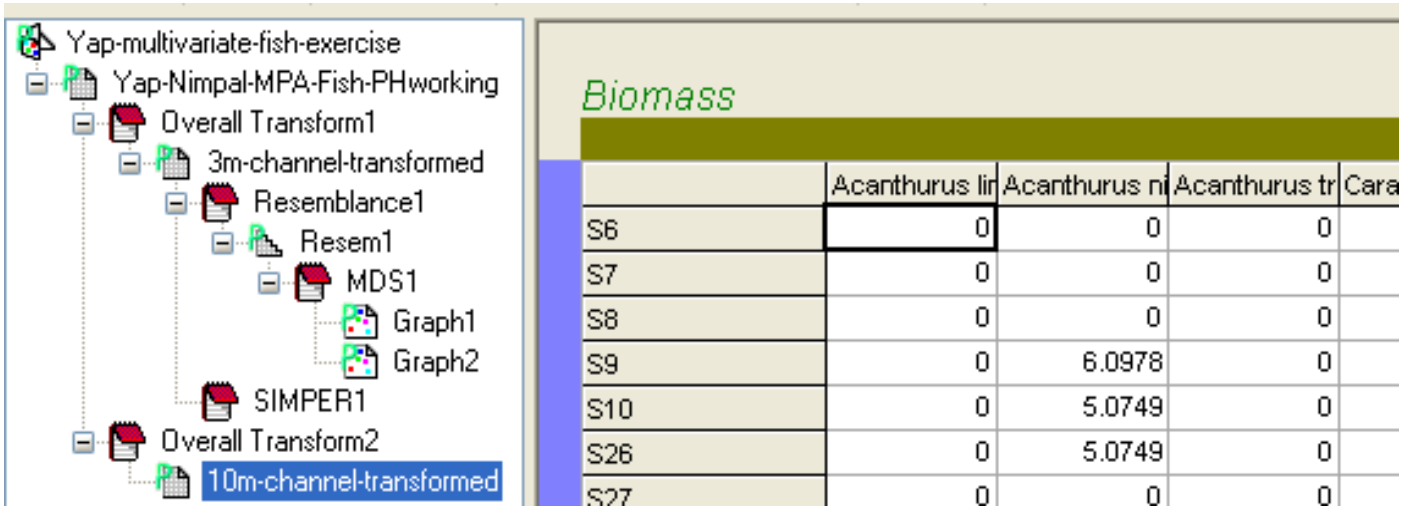
59. Select the "***Analyze***" menu,
- **a. Go to** "***pre-treatment***",
- **b. Select** "***Transform overall***".

60. **Select** "***Log (X+1)***" from the drop down menu and
61. Click OK.

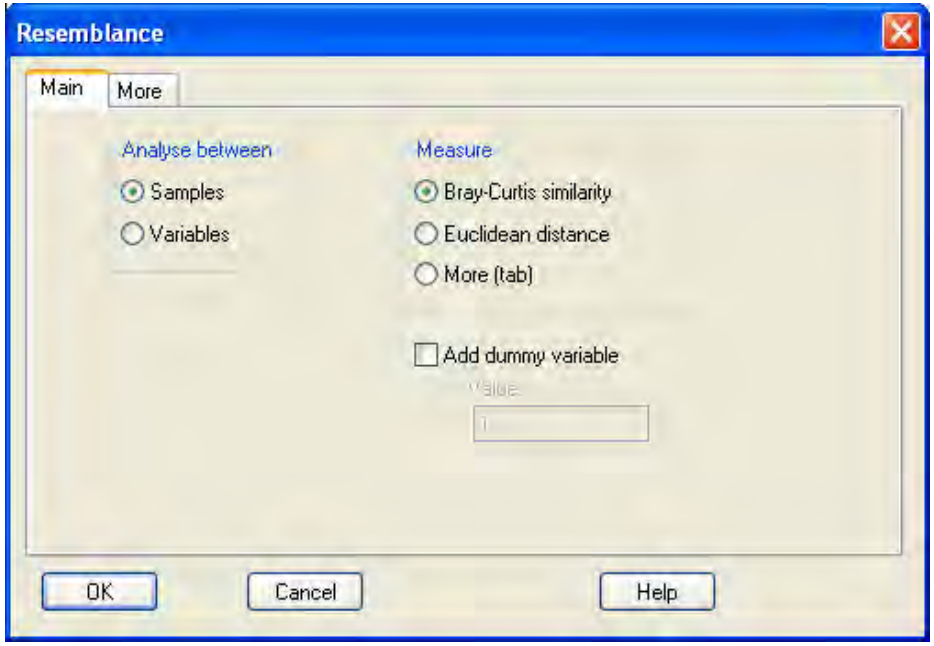


You have now created a new species by site datasheet, you can see on the left that the current name is "Data1".

62. **Rename** this to "***10m-channel-transformed***".



63. **Go to** the "***Analyze***" menu
    a. **Select** "***Resemblance***".



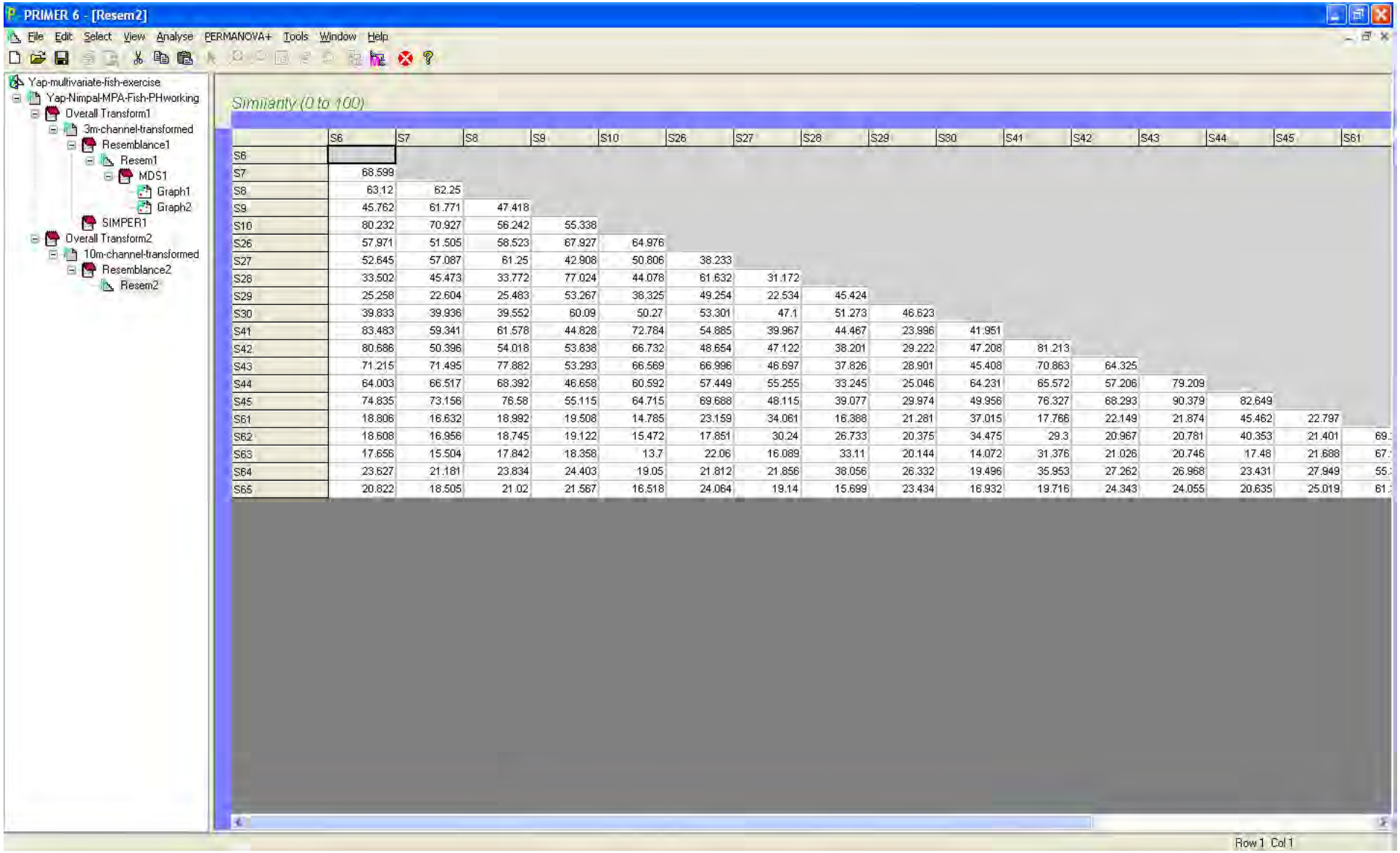
64. Under "***Analyze Between***"

- **a. Select** "Samples"

65. Under "***Measure*****"**

- **a. Select** "***Bray-Curtis similarity***".

66. Click OK.

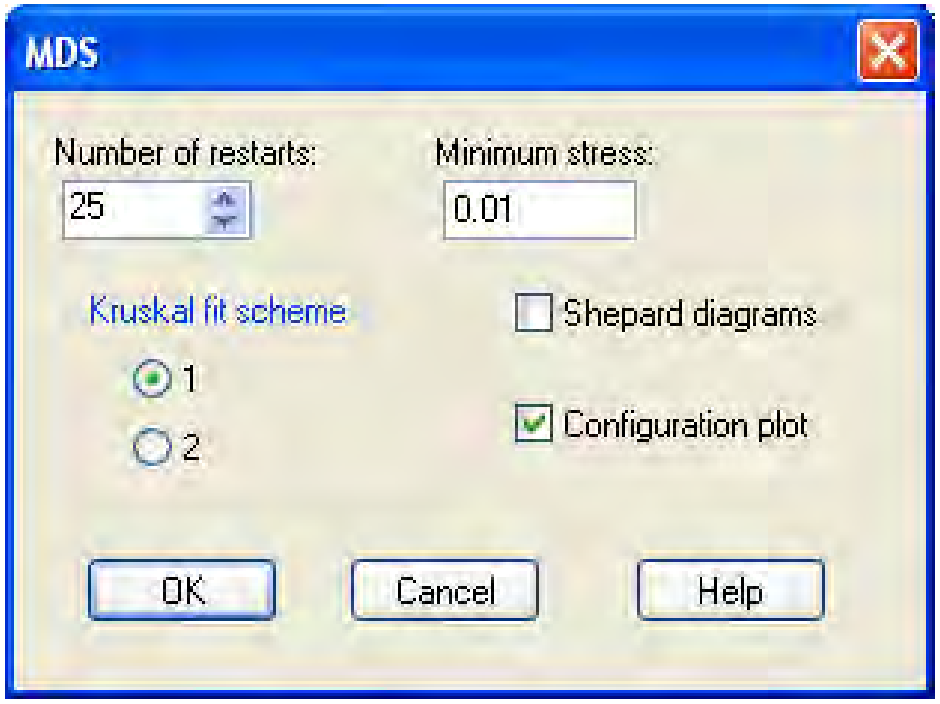


	S6	S7	S8	S9	S10	S26	S27	S28	S29	S30	S41	S42	S43	S44	S45	S61
S6																
S7	68.599															
S8	63.12	62.25														
S9	45.762	61.771	47.418													
S10	80.232	70.927	56.242	55.338												
S26	57.971	51.505	58.523	67.927	64.976											
S27	52.645	57.087	61.25	42.908	50.806	38.233										
S28	33.502	45.473	33.772	77.024	44.078	61.632	31.172									
S29	25.258	22.604	25.483	53.267	36.325	49.254	22.534	45.424								
S30	39.833	39.936	39.552	60.09	50.27	53.301	47.1	51.273	46.623							
S41	83.483	59.341	61.578	44.828	72.784	54.885	39.967	44.467	23.996	41.951						
S42	80.686	50.396	54.018	53.838	66.732	48.654	47.122	38.201	29.222	47.208	81.213					
S43	71.215	71.495	77.882	53.293	66.569	66.996	46.697	37.826	28.901	45.408	70.863	64.325				
S44	64.003	66.517	68.392	46.658	60.592	57.449	55.255	33.245	25.046	64.231	65.572	57.206	79.209			
S45	74.835	73.156	76.58	55.115	64.715	69.688	48.115	39.077	29.974	49.956	76.327	68.293	90.379	82.649		
S61	18.806	16.632	18.992	19.508	14.785	23.159	34.061	16.388	21.281	37.015	17.766	22.149	21.874	45.462	22.797	
S62	18.608	16.956	18.745	19.122	15.472	17.851	30.24	26.733	20.375	34.475	29.3	20.967	20.781	40.353	21.401	69.
S63	17.656	15.504	17.842	18.358	13.7	22.06	16.089	33.11	20.144	14.072	31.376	21.026	20.746	17.48	21.688	67.
S64	23.627	21.181	23.834	24.403	19.05	21.812	21.856	38.056	26.332	19.496	35.953	27.262	26.968	23.431	27.949	55.
S65	20.822	18.505	21.02	21.567	16.518	24.064	19.14	15.699	23.434	16.932	19.716	24.343	24.055	20.635	25.019	61.

Now we have a “data matrix” that compares every possible combination of transects, and provides a distance measure of ecological similarity for each comparison. From this we will again create our multi-dimensional scaling plot (MDS plot).

67. Go to the “***Analyze***” menu.

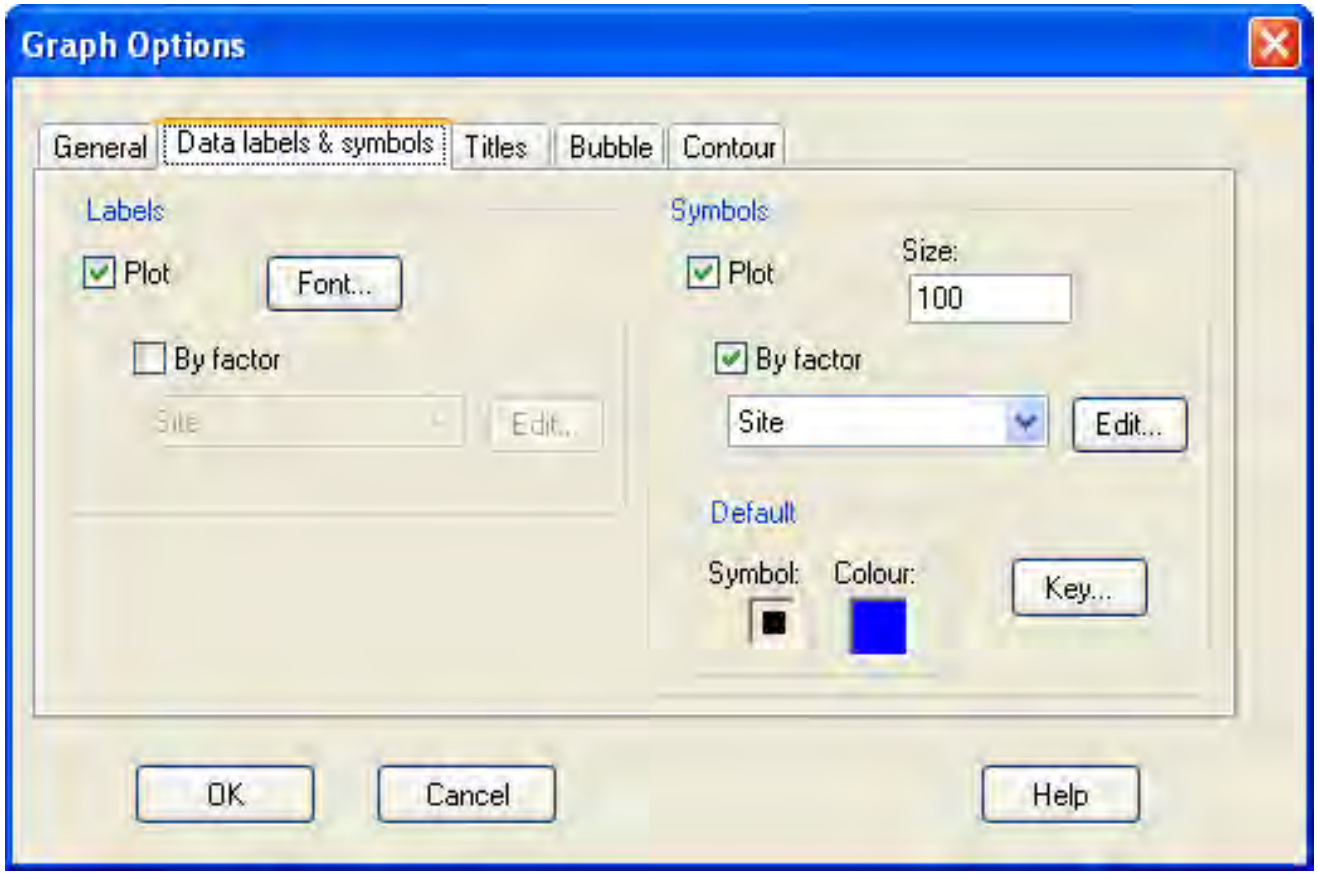
68. Select MDS.



a. Keep the default settings for our options
b. Click OK.

After a bit of processing time, PRIMER again produces the 2-dimensional and 3 dimensional plots called "Graph1 and Graph2". Let's just focus on the first, 2-dimensional plot.

69. Under the "***Graph***" menu,
70. Select "***Data labels & symbols***".



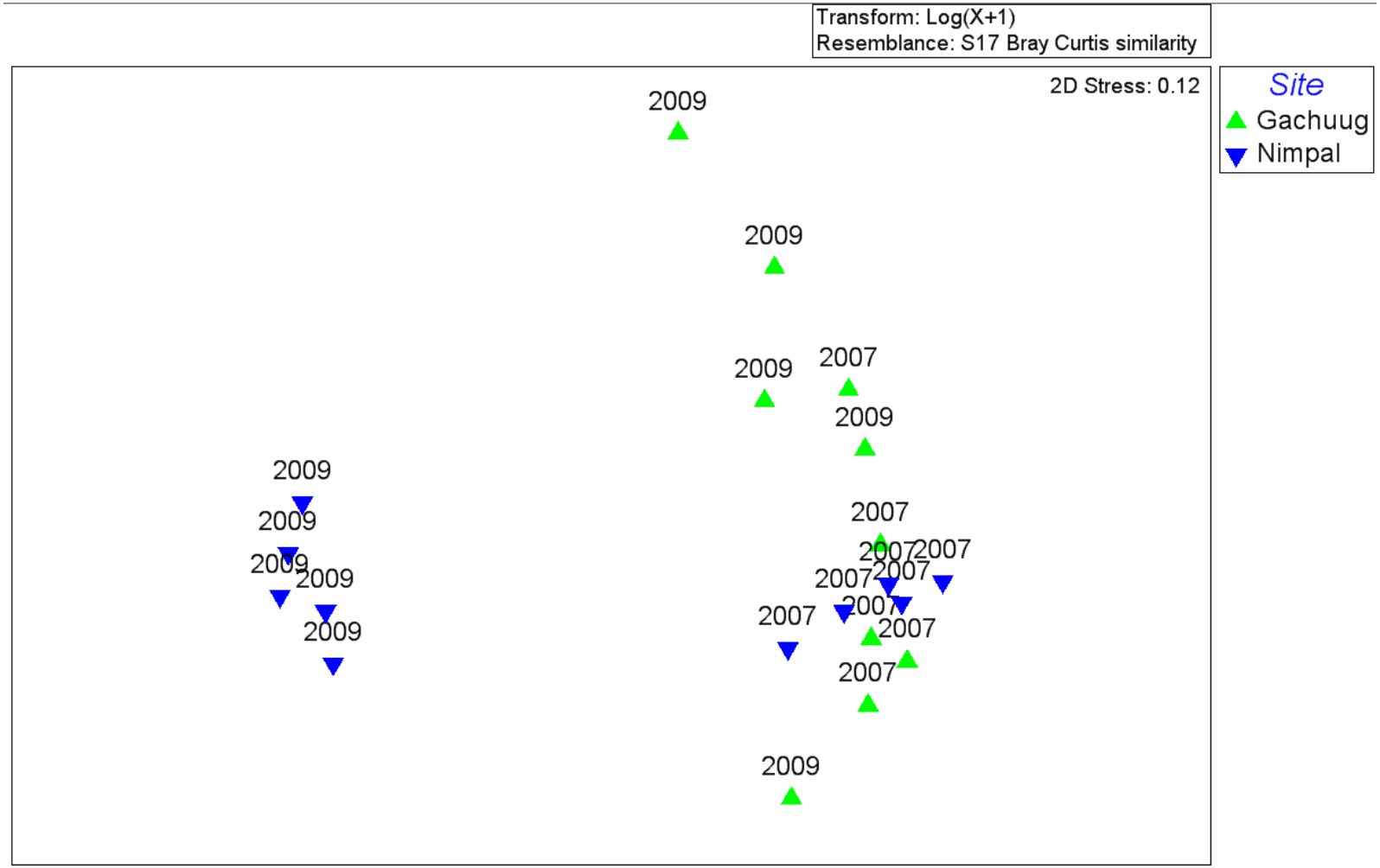
71. For "***Labels***"

- **a. Check** the "By factor" box
- **b.** From the drop down menu **select** "***Year***".

72. For "***Symbols***"

- **a. Check** the "***By factor***" box
- **b.** From the drop down menu **select** "***Site***".

73. You should have changed the look of your graph, confirm.



Notice we have very similar trends compared with our 3m depth analyses earlier.

Next we will calculate the contribution of each species of fish to our detected trends using the SIMPER analyses again.

74. Go back to the "***10m-channel-transformed' data sheet***"

We need to make further selections from our data. What we want to know is how and why the fish biomass are different between these reefs in 2009 only, because in 2007 they were still similar. Basically, we'd like to know what change occurred.

75. Select the "***Edit***" menu
   a. Select "***Factors***".

Notice only our subset of sites appears. For our next examination we wish to look at only 2009 data, corresponding to samples (***S21-25***) and (***S56-60***).

Note those sample labels on your scratch paper and close the factors box.

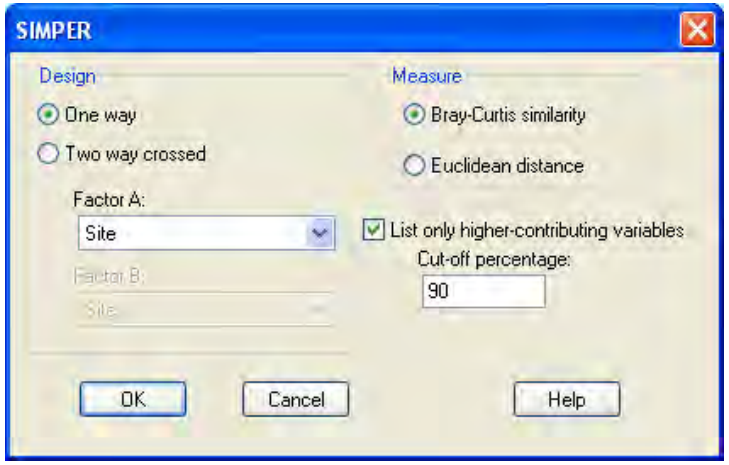
76. On your main sheet
   a. Highlight the samples noted above,
77. Go to the "***Select***" menu
   a. Select "***highlighted***".
78. Confirm your datasheet below.

Notice only 10 samples remain, these correspond to 10 transects surveyed, 5 inside of the MPA at a 10m depth in 2009, and 5 outside.

Biomass

	Variables															
	Acanthurus lir	Acanthurus ni	Acanthurus tr	Caranx melam	Cephalopholus	Cheilinus undu	Chlorurus mic	Chlorurus sor	Ctenochaetus	Epinephelus m	Epinephelus m	Grouper	Hipposcarus l	Kyphosus	Lutjanus gibbu	Lutjanus m
S26	0	5.0749	0	0	0	0	0	4.6195	4.186	0	0	0	0	0	0	
S27	0	0	0	0	0	0	0	5.6895	5.518	0	3.0177	0	5.6386	0	3.736	
S28	0	6.9327	0	0	5.4169	4.1945	0	5.0327	3.324	0	0	0	0	0	0	
S29	0	7.241	0	0	0	0	7.3037	0	5.7143	0	0	0	0	0	0	
S30	0	7.1062	0	0	0	0	0	6.8617	6.6336	0	0	0	0	0	6.0075	
S61	0	0	0	0	0	0	0	0	3.8227	0	0	0	0	0	5.5061	
S62	0	0	0	0	3.9545	0	0	0	5.0746	0	0	6.2201	0	0	6.5396	
S63	0	0	0	0	2.9997	0	0	0	3.3798	0	0	0	0	0	0	
S64	0	0	0	0	8.1371	0	0	0	5.437	0	0	0	0	0	0	
S65	0	0	0	0	0	0	0	0	4.4177	0	0	5.7634	0	0	0	

79. Go to the "***Analyze***" menu
   a. Select "SIMPER" (which is short for analyses of similarities).



80. Under "***Factor A***:"

- **a. Select** "***Site***" from the drop down menu

So we can determine differences can leave the default settings that match our MDS plot generation, and

81. Click OK.

82. Confirm.

Groups Gachuug & Nimpal
Average dissimilarity = 76.25

	Group Gachuug	Group Nimpal				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Scarus sp.	0.00	7.15	16.50	5.96	21.64	21.64
Acanthurus nigricauda	5.27	0.00	12.37	1.82	16.23	37.86
Chlorurus sordidus	4.44	0.00	9.83	1.89	12.90	50.76
Cephalopholus argus	1.08	3.02	6.97	1.07	9.15	59.90
Lutjanus gibbus	1.95	2.41	6.18	1.01	8.11	68.01
Grouper	0.00	2.40	5.06	0.78	6.64	74.65
Chlorurus microrhinos	1.46	0.00	3.75	0.48	4.92	79.57
Macolor macularis	1.63	0.00	3.03	0.49	3.98	83.55
Ctenochaetus striatus	5.08	4.43	2.89	1.45	3.79	87.35
Hipposcarus longiceps	1.13	0.00	2.37	0.49	3.11	90.46

Scroll down the text output sheet so we can see the comparison between the two sites. The relevant section was again manually highlighted in blue for identification.

From this table three columns are most informative. The first column has the average biomass from Gachuug (the reference site) for each fish species. The second has data from Nimpal. For now, we continue to disregard the next two columns and focus upon the % contribution. We are most interested in what species contributed to the majority of the difference found in our MDS plot. Notice the first three fish cumulatively accounted for > 50% of the variance (the last column tells us the cumulative variance accounted for). So we should logically focus upon these three species. The most notable difference, again, is a shift in parrotfish from Chlorurus sordidus, very common at the reference site, to 'other Scarids' (including a mixture of other species of parrotfish besides the common ones, as noted by Yap's monitoring program). Also, there has been an increase in the grouper (Cephalopholus argus).

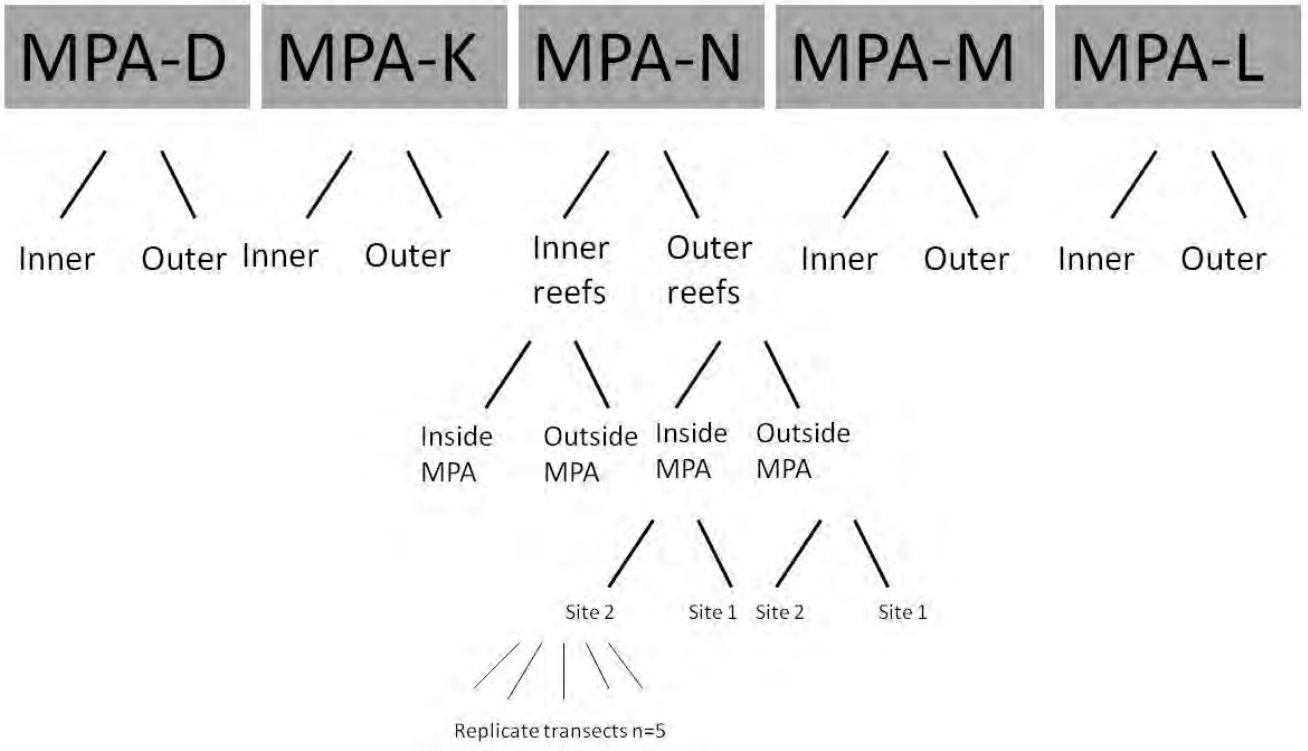
We could continue to do this for the "Outer" reefs too, but for our purposes we can conclude the exercise now. We conclude that substantial changes ***appear*** *to have occurred between 2007 and 2009 for the "Channel" monitoring sites associated with Nimpal MPA and Gachuug reference area. In a later exercise we will test whether or not these changes were* ***statistically significant*** *using a multivariate, nested ANOVA approach. This exercise was intended to improve our ability to visualize and comprehend our data initially. Often we'd like to have immediate insight into potential trends, regardless of statistical significance, soon after our surveys are conducted. This exercise represents one means at gaining quick insight into multivariate patterns in our collected ecological datasets.*

End of Exercise 7

Exercise 8 – A multivariate, statistical examination of Pohnpei's Marine Protected Areas using PRIMER-E and PERMANOVA+

For this exercise we will refer back to the Pohnpei marine protected area fish biomass data we began to explore in exercise 3 and 4. We will be looking these data from a multivariate perspective in order to understand the status of each MPA. More formally, we will examine how the variance in the fish dataset is spread out among the numerous independent variables that emerge from their monitoring program design. To this end, we will also test for statistical significance, providing a guide for future work with datasets of your choosing.

It will help to examine a diagram of the survey design used. There were five villages that have established MPA's within them, noted as D, K, N, M, and L. Each MPA encompasses both inner lagoon and outer reef sampling sites. For each reef type two sampling sites were set up inside and outside the MPA. Finally, at each sampling site there were 5 transects surveyed.



This type of experimental design is defined as "nested". Reef type is nested within village location, MPA status is nested within reef type, and sites are nested within each MPA status. Using this design we can examine all MPA's together or individually, however it is always best to start our investigations with a big-picture perspective (i.e., highest levels first), then work our way down based upon significant findings. We are interested in determining what nesting level, or levels, explain significant proportions of the variation in the fish biomass data, obviously we are most interested in learning about MPA status, but we wish to account for all other predictable variation that is possible to do so. The PERMANOVA+ software allows us to efficiently do this for our multivariate dataset.

First we need to import our data from excel as we did in the previous exercise.

1. **Open** the "***Pohnpei-MPA-fish-PERMANOVA-example***" file.

Take a look at both worksheets. First look at the "**Data**" sheet. You can see the metadata columns follow the diagram above, starting with '**Location'** and ending with '**Transect #**'. After these information data you can see each indicator fish species, and the biomass.

2. **Click on** the sheet "***For Primer***".

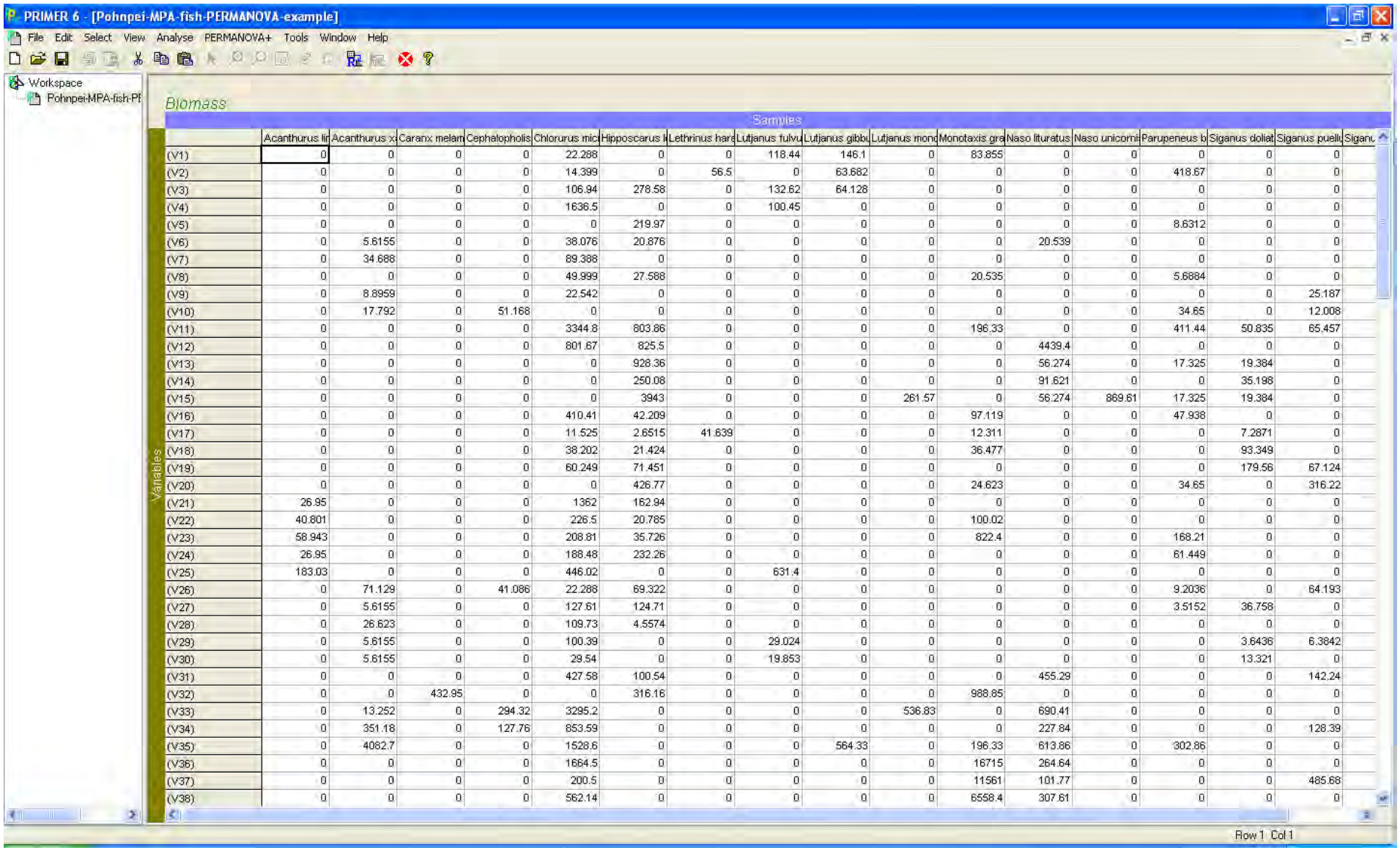
These are the same data arranged for input into PRIMER to import the numerical data, and the explanatory factors. You can see the fish abundance data appear first, but as you scroll to the right you eventually come to a blank row, then the informational data. This is the format that is required by PRIMER, used in our previous exercise. Numerical data followed by a blank, then categorical data.

3. **Close** Excel and **Open** PRIMER.
4. **Select *Open*** from the menu.
   a. **Select** Excel under the ***dropdown menu*** for '***files of type'***
   b. **Navigate** to "***Pohnpei-MPA-fish-PERMANOVA-example.xlsx***"
   c. **Click** "Open".

Note that PERMANOVA stands for "permutation multivariate ANOVA".

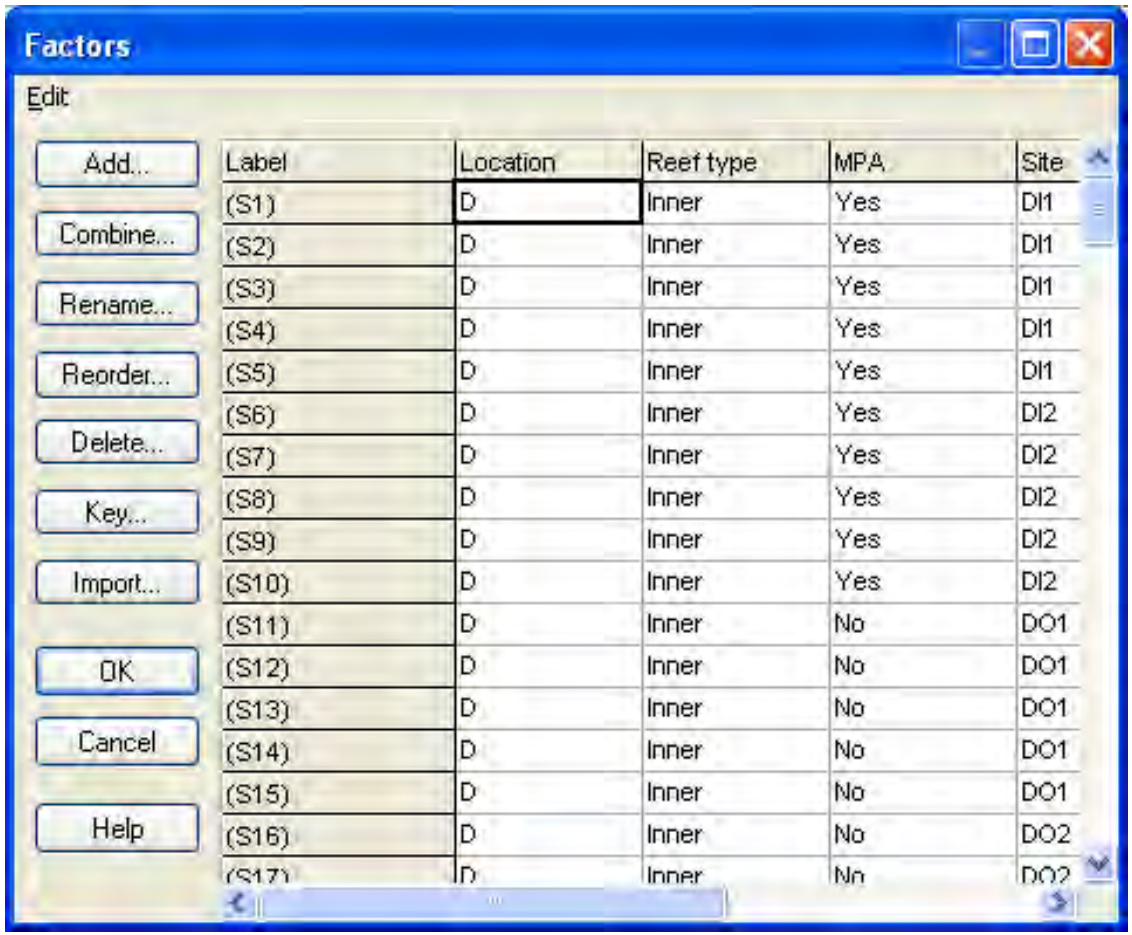
5. In the next menu box
   a. **Click** the dropdown menu and **choose** the excel worksheet titled "***For Primer***".
   b. **Select** "***Sample data***" as the data type
   c. **Click** next.
6. **Uncheck** the two green marks next to "***Title***" and "***Row labels***" (*we do not have either of these in our Excel file*)
   a. **Select** "***Samples as rows***" for the Orientation.
   b. **Select** "***Biomass***" for the data type.
   c. **Click** Finish.

7. Confirm below.



***Note**: Check to ensure that the "factors" have all been imported too.*

8. Under the '***Edit'*** menu
   a. **Select** "***factors***"
9. Confirm below.



You can see that all of our factors have been automatically imported by PRIMER. We are going to use a useful feature in PRIMER and make a new factor that is a combination of several of the others. This will be done so we can generate a better graphical interpretation at a later stage in this exercise.

10. **Click** on the "***Combine***" box on the left.
    a. **Place** '***Location'***, '***Reef type'***, '***MPA'***, and '***Site'*** in the "***Include***" box ***in that order***, which follows our experimental design diagram above.
    b. **Click** OK.

You can see your new factor has appeared.

11. Using the rename box on the left,
    a. **Rename** this factor to "***Combined name***".
    b. **Click** OK (***Note****: No changes will be saved unless you click on OK*)

In order to help us set up our PERMANOVA design, let's first gain a big-picture perspective of the data set. To do this we will create a multi-dimensional scaling plot, similar to the last exercise.

12. **Go to** the "Analyze" menu
    a. **Select** "***pre-treatment***".
    b. **Select** "***transform overall***"
13. In the ***dropdown menu***
    a. **Select** "***Log(x+1)***".

b. Click OK.

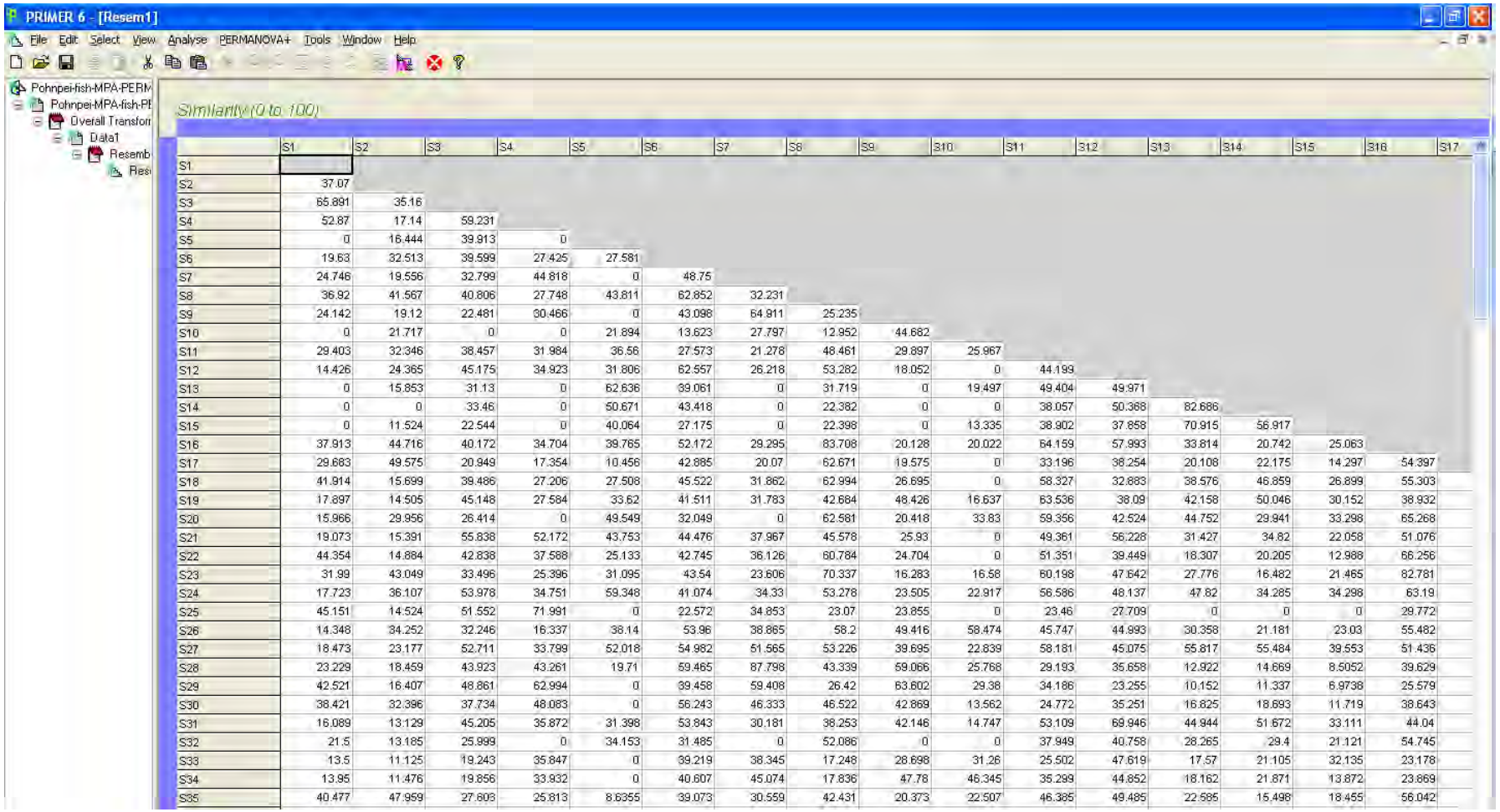
A new sheet with the log-transformed data should appear.

14. Go to the “Analyze” menu

a. Create a ***Bray-Curtis*** similarity matrix by scrolling down to “***resemblance***” (*make sure the analyses is between samples and you use a Bray-Curtis similarity method*)

15. Click OK

16. Confirm.



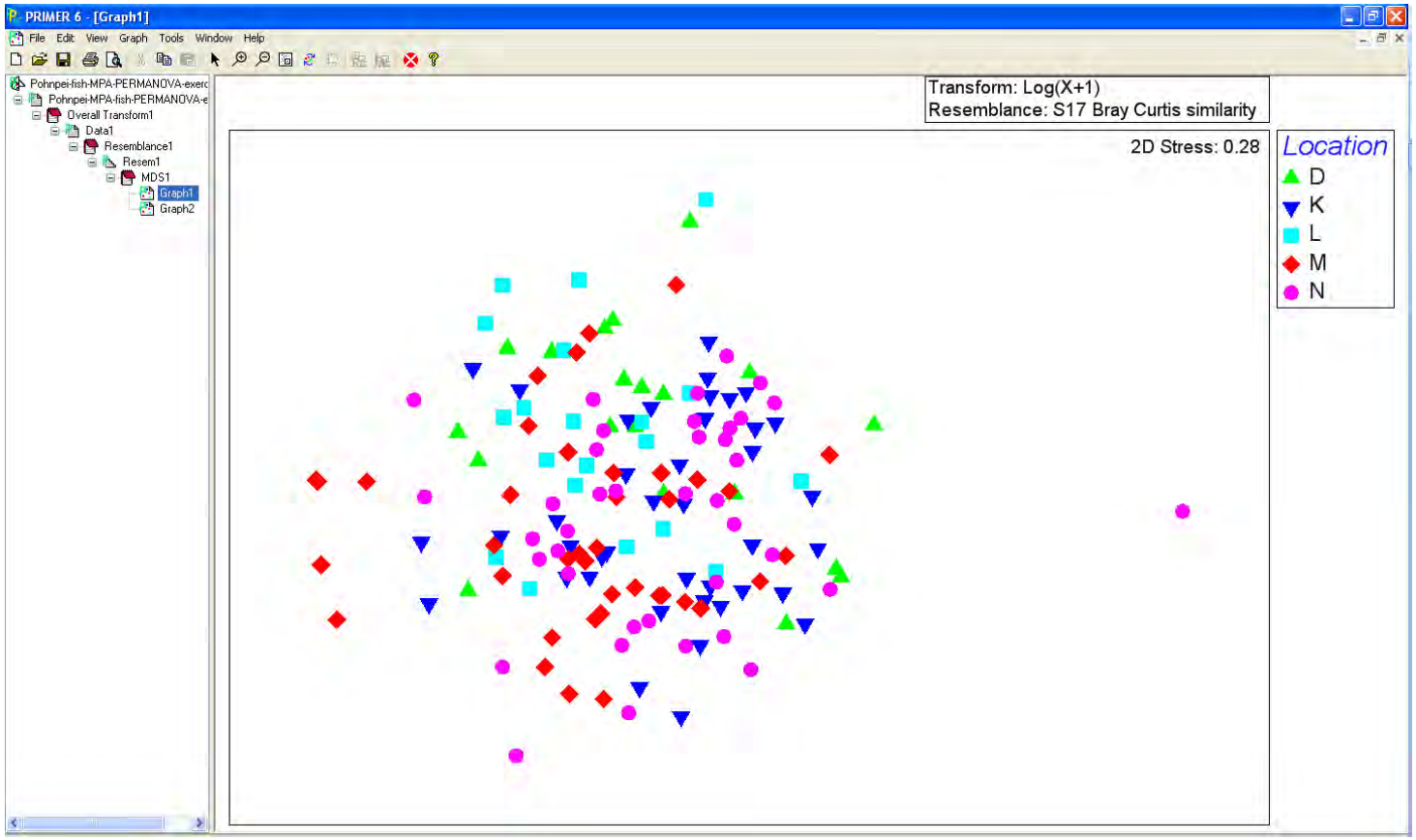
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
S1																	
S2	37.07																
S3	65.891	35.16															
S4	52.87	17.14	59.231														
S5	0	16.444	39.913	0													
S6	19.63	32.513	39.599	27.425	27.581												
S7	24.746	19.556	32.799	44.818	0	48.75											
S8	36.92	41.567	40.806	27.748	43.811	62.852	32.231										
S9	24.142	19.12	22.481	30.466	0	43.098	64.911	25.235									
S10	0	21.717	0	0	21.894	13.623	27.797	12.952	44.682								
S11	29.403	32.346	38.457	31.984	36.56	27.573	21.278	48.461	29.897	25.967							
S12	14.426	24.365	45.175	34.923	31.806	62.557	26.218	53.282	18.052	0	44.199						
S13	0	15.853	31.13	0	62.636	39.061	0	31.719	0	19.497	49.404	49.971					
S14	0	0	33.46	0	50.671	43.418	0	22.382	0	0	38.057	50.368	82.686				
S15	0	11.524	22.544	0	40.064	27.175	0	22.398	0	13.335	38.902	37.858	70.915	56.917			
S16	37.913	44.716	40.172	34.704	39.765	52.172	29.295	83.708	20.128	20.022	64.159	57.993	33.814	20.742	25.063		
S17	29.683	49.575	20.949	17.354	10.456	42.885	20.07	62.671	19.575	0	33.196	38.254	20.108	22.175	14.297	54.397	
S18	41.914	15.699	39.486	27.206	27.508	45.522	31.862	62.994	26.695	0	58.327	32.883	38.576	45.859	26.899	55.303	
S19	17.897	14.505	45.148	27.584	33.62	41.511	31.783	42.684	48.426	16.637	63.536	38.09	42.158	50.046	30.152	38.932	
S20	15.966	29.956	26.414	0	49.549	32.049	0	62.581	20.418	33.83	59.356	42.524	44.752	29.941	33.298	65.268	
S21	19.073	15.391	55.838	52.172	43.753	44.476	37.967	45.578	25.93	0	49.361	56.228	31.427	34.82	22.058	51.076	
S22	44.354	14.884	42.838	37.588	25.133	42.745	36.126	60.784	24.704	0	51.351	39.449	18.307	20.205	12.988	66.256	
S23	31.99	43.049	33.496	25.396	31.095	43.54	23.606	70.337	16.283	16.58	60.198	47.642	27.776	16.482	21.465	82.781	
S24	17.723	36.107	53.978	34.751	59.348	41.074	34.33	53.278	23.505	22.917	56.586	48.137	47.82	34.285	34.298	63.19	
S25	45.151	14.524	51.552	71.991	0	22.572	34.853	23.07	23.855	0	23.46	27.709	0	0	0	29.772	
S26	14.348	34.252	32.246	16.337	38.14	53.96	38.865	58.2	49.416	58.474	45.747	44.993	30.358	21.181	23.03	55.482	
S27	18.473	23.177	52.711	33.799	52.018	54.982	51.565	53.226	39.695	22.839	58.181	45.075	55.817	55.484	39.553	51.436	
S28	23.229	18.459	43.923	43.261	19.71	59.465	87.798	43.339	59.066	25.768	29.193	35.658	12.922	14.669	8.5052	39.629	
S29	42.521	16.407	48.861	62.994	0	39.458	59.408	26.42	63.602	29.38	34.186	23.255	10.152	11.337	6.9738	25.579	
S30	38.421	32.396	37.734	48.083	0	56.243	46.333	46.522	42.869	13.562	24.772	35.251	16.825	18.693	11.719	38.643	
S31	16.089	13.129	45.205	35.872	31.398	53.843	30.181	38.253	42.146	14.747	53.109	69.946	44.944	51.672	33.111	44.04	
S32	21.5	13.185	25.999	0	34.153	31.485	0	52.086	0	0	37.949	40.758	28.265	29.4	21.121	54.745	
S33	13.5	11.125	19.243	35.847	0	39.219	38.345	17.248	28.698	31.26	25.502	47.619	17.57	21.105	32.135	23.178	
S34	13.95	11.476	19.856	33.932	0	40.607	45.074	17.836	47.78	46.345	35.299	44.852	18.162	21.871	13.872	23.869	
S35	40.477	47.959	27.603	25.813	8.6355	39.073	30.559	42.431	20.373	22.507	46.385	49.485	22.585	15.498	18.455	56.042	

Now we just need to create multi-dimensional scaling plot to improve our big-picture understanding before moving forward.

17. **Go to** "***Analyse***",
    a. **Select** "***MDS***" (*wait for the computer to process the required calculations*)

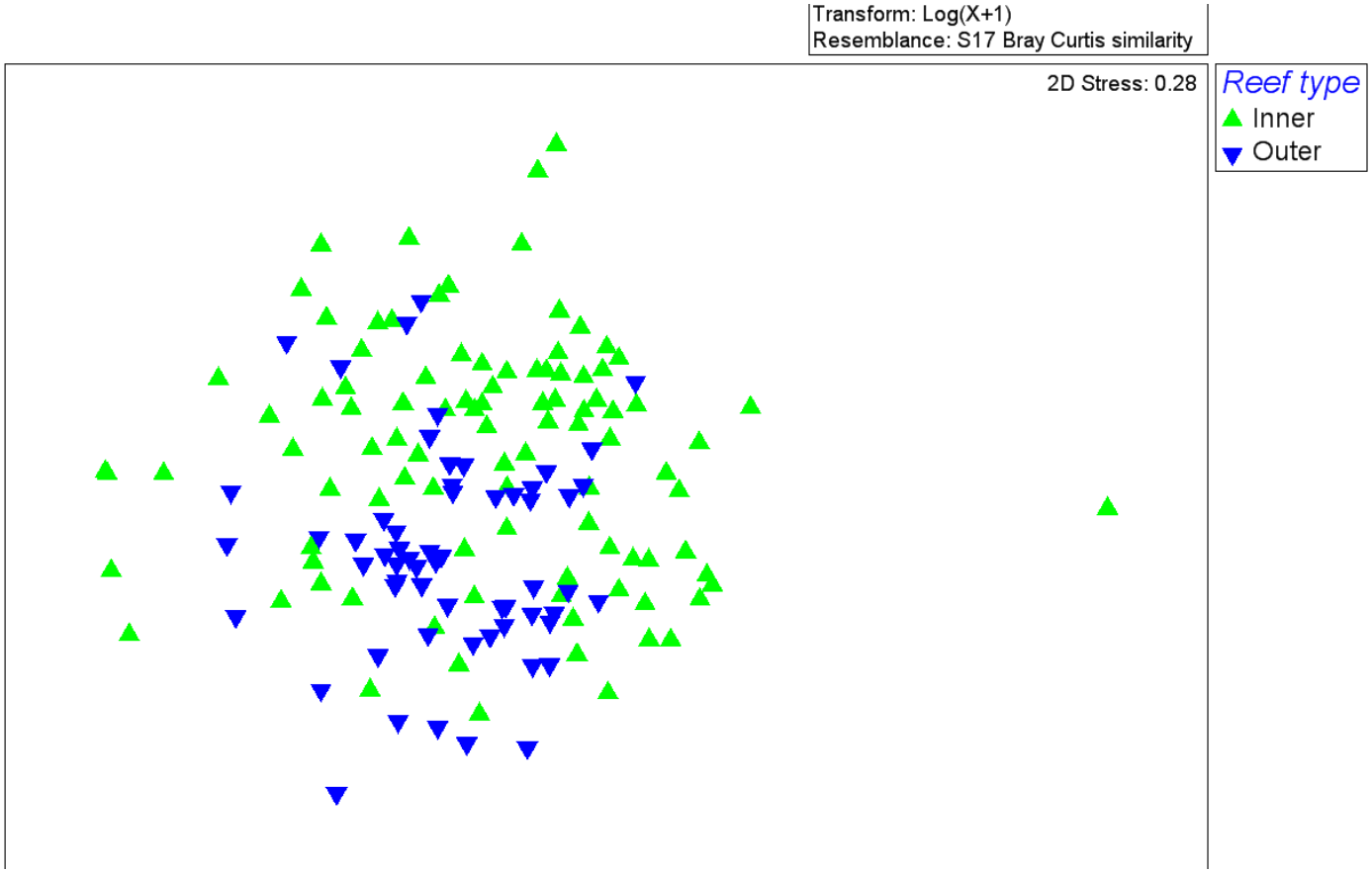
Once completed lets change the look of the graph to gain a better perspective for our analyses.

18. **Go to** the "***Graph***" menu
    a. **Select** "***Data Labels & Symbols***".
    b. On the "***Labels***" left hand side **uncheck** the box that says "***Plot***".
    c. On the "***Symbols***" side **change** the factor dropdown menu to "***Location***".
19. **Click** OK
20. Confirm.



This MDS plot shows similarities between the individual fish transect data from each location, but it does not tell us anything about the different reef types, MPA status, or individual sites yet. The intermixing of symbols and colors strongly suggests that there are no strong difference in the overall composition of fish assemblages between the locations. Lets look at the reef types.

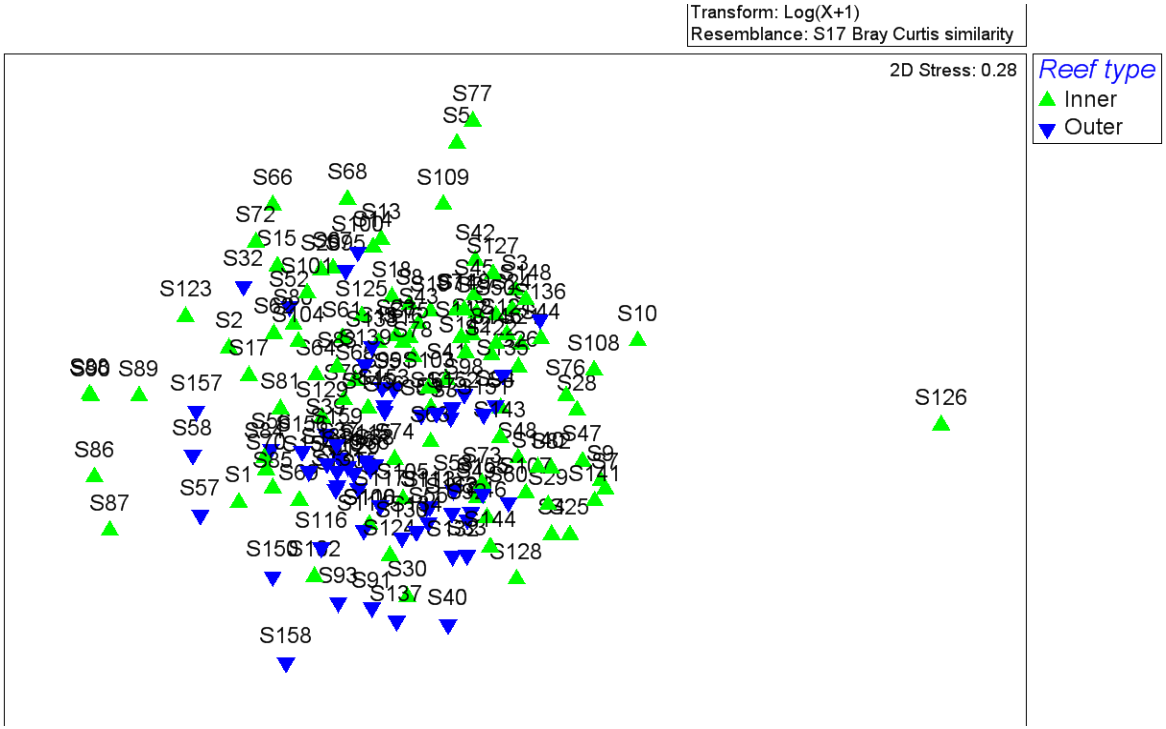
21. **Go to** the "***Graph***" main menu and
    a. **Select** "***Data Labels & Symbols***"
22. On the "***Symbols***" side
    a. **Change** the factor dropdown menu to "***Reef type***".
23. **Click** OK
24. Confirm.



In this MDS plot we can start to see where some of the ecological variation exists. While not extremely clear, we can start to see separation between the two different reef types, regardless of MPA status. This tells us that in order to compare MPA and reference sites, it would be a very good idea to first account for reef type, which we will do next. Before moving on you can change the data symbols to other factors if you like. One last note here, there is one green triangle in the far right hand of the above plot. These seems to be a strong outlier, meaning it is very unlike any of the other transects. Typically when this occurs there may have been an error in the data collection or entry, or this may just be a very unique situation. Either way, we should remove this outlier point from further analyses, as it may bias the outcome.

Now let's find out the name of the outlier sample.

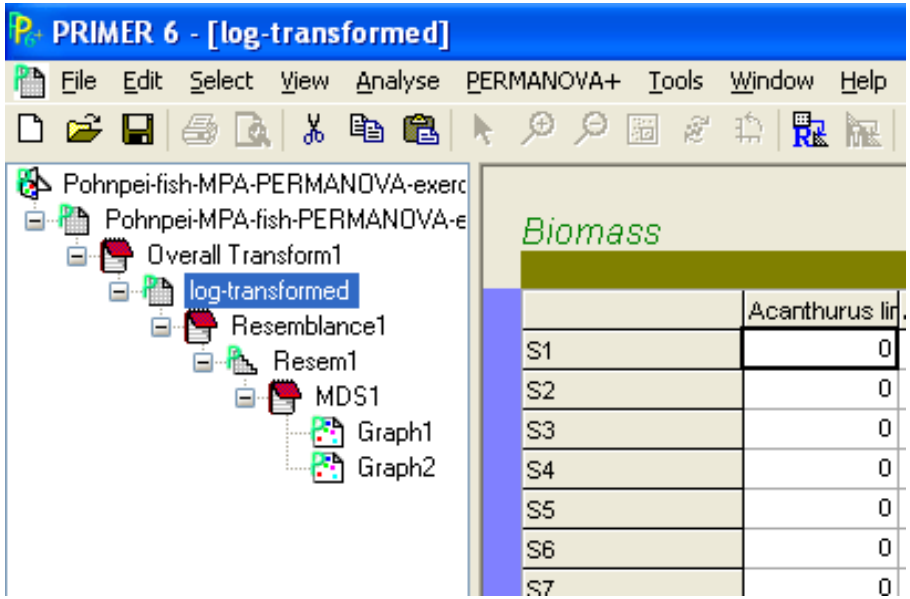
25. **Go back** to the "***Graph***" menu
    a. **Select** "***Data Labels & Symbols***"
26. On the left, **click** on box for "***Plot***" the labels.
27. **Click** OK
28. Confirm.



We can see that our outlier transect is “S126”. Note that on your scratch paper.

29. Move back to our data file, after the log transformation.
- **a.** On the left, **make active** the “***Data1***” sheet.
- **b.** **Rename** to “***log-transformed***”.

30. Confirm.



Now with this sheet active

31. **Highlight** all the data by **clicking** in the box **above** “***S1***” and to the **left** of “***Acanthurus lineatus***”.

The data sheet should change color.

32. **Scroll down** to “***S126***”
- **a.** **Click** on that row.

That row should change to a different color.

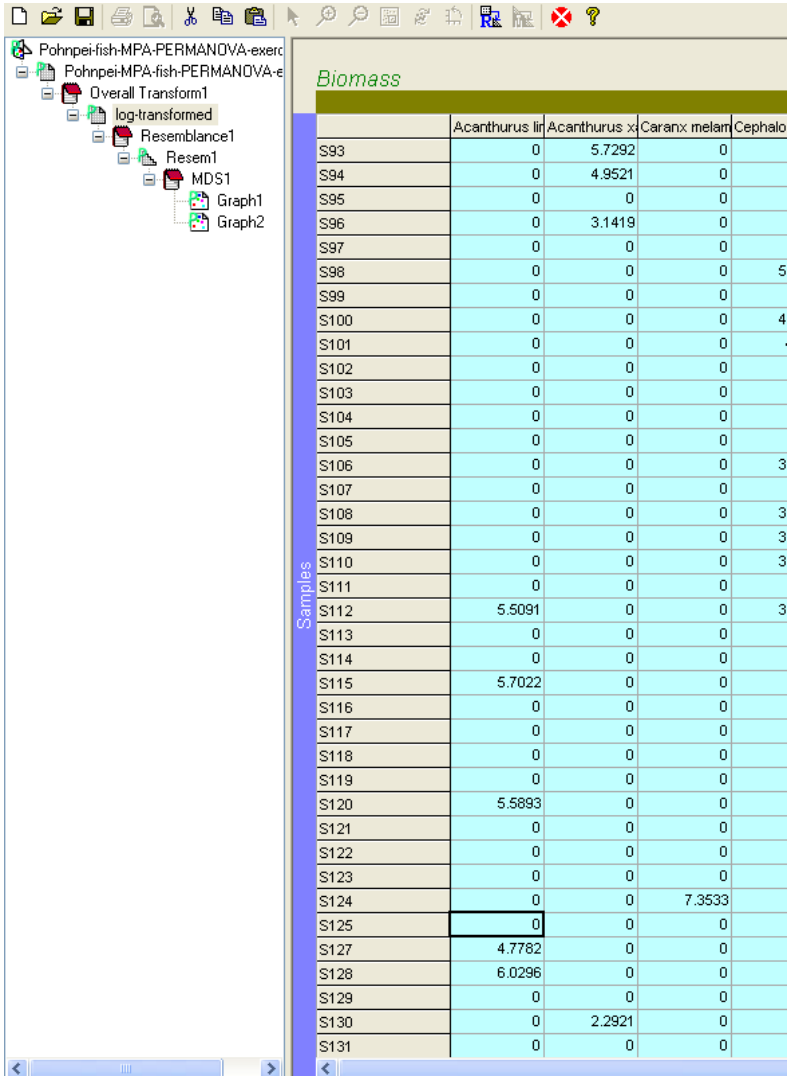
33. Go to the "***Select***" menu on top

a. Scroll down to "***Highlighted***".

Now you have a new datasheet with the outlier data removed, ready for further analyses.

Note*: Check to ensure that S126 is no longer there.*

34. Confirm.



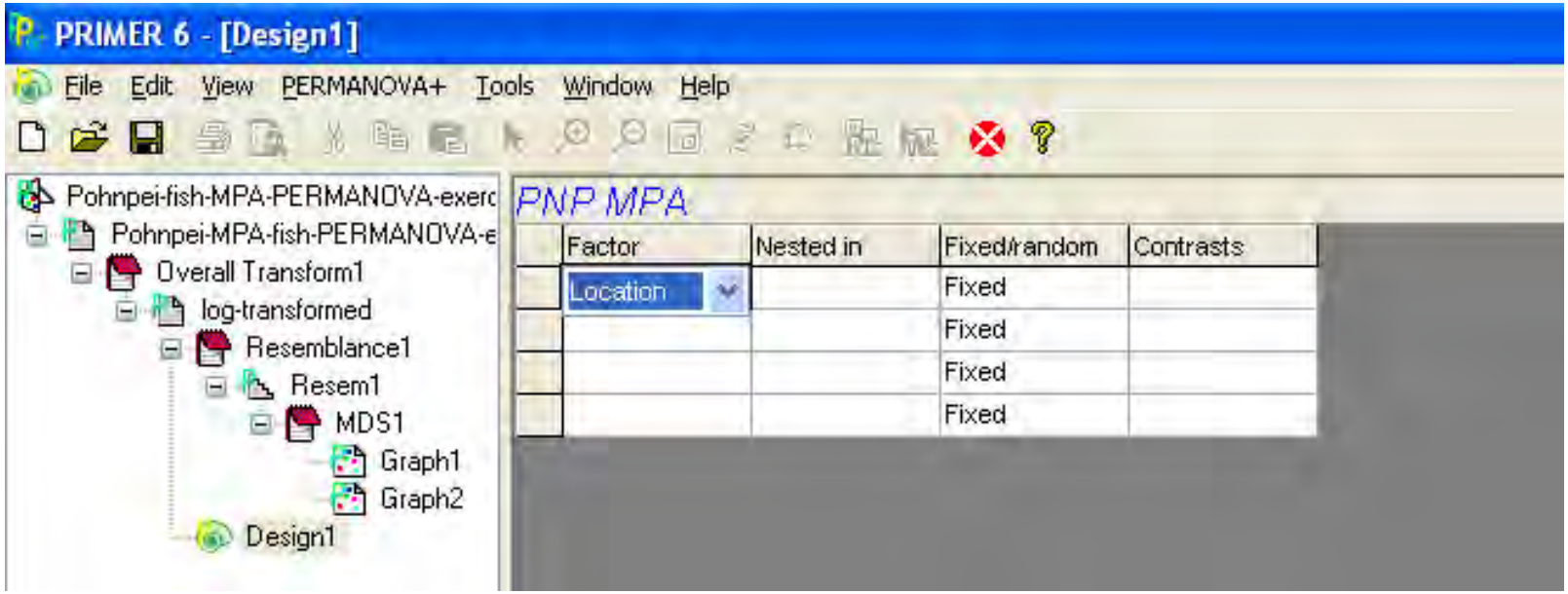
	Acanthurus lin	Acanthurus x	Caranx melam	Cephalo
S93	0	5.7292	0	
S94	0	4.9521	0	
S95	0	0	0	
S96	0	3.1419	0	
S97	0	0	0	
S98	0	0	0	5
S99	0	0	0	
S100	0	0	0	4
S101	0	0	0	
S102	0	0	0	
S103	0	0	0	
S104	0	0	0	
S105	0	0	0	
S106	0	0	0	3
S107	0	0	0	
S108	0	0	0	3
S109	0	0	0	3
S110	0	0	0	3
S111	0	0	0	
S112	5.5091	0	0	3
S113	0	0	0	
S114	0	0	0	
S115	5.7022	0	0	
S116	0	0	0	
S117	0	0	0	
S118	0	0	0	
S119	0	0	0	
S120	5.5893	0	0	
S121	0	0	0	
S122	0	0	0	
S123	0	0	0	
S124	0	0	7.3533	
S125	0	0	0	
S127	4.7782	0	0	
S128	6.0296	0	0	
S129	0	0	0	
S130	0	2.2921	0	
S131	0	0	0	

Now, we are ready to design our PERMANOVA+ analysis. This is just like designing any standard ANOVA analysis.

35. **Go to** the ***PERMANOVA***+ menu
36. **Select** "***Create PERMANOVA+ design***".
37. **Title** this "***PNP MPA***".

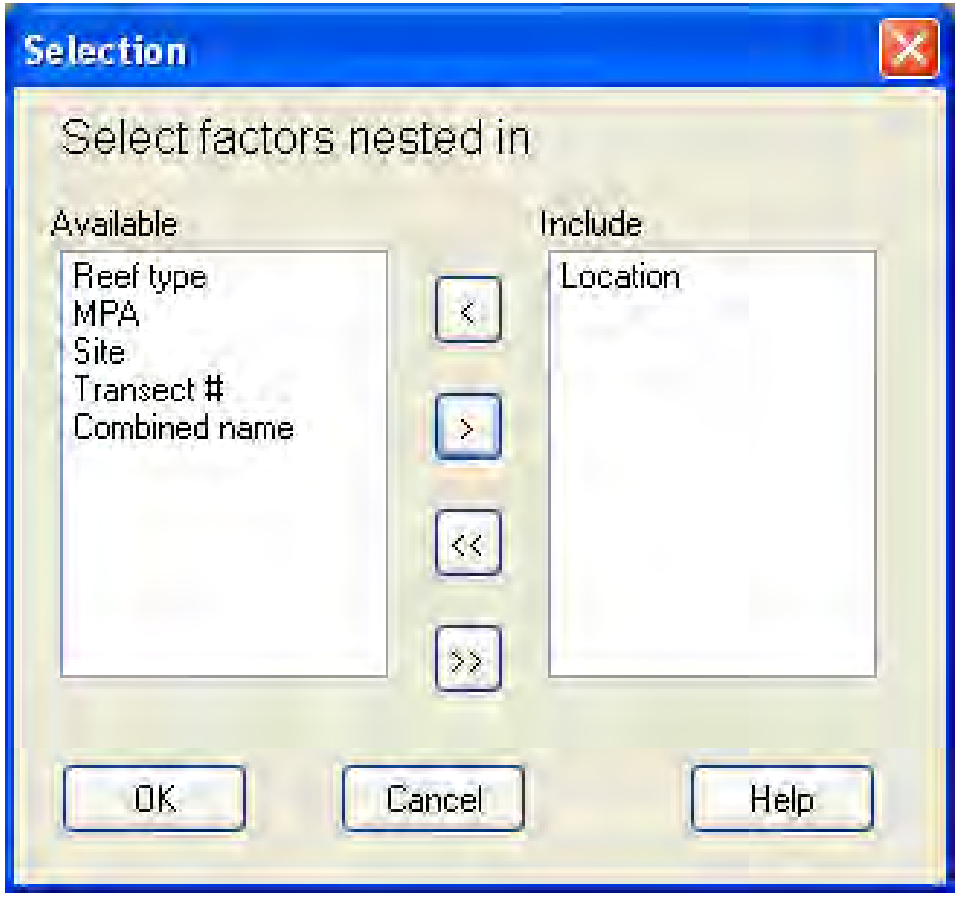
Recall from our diagram above we have four factors: 1) Location, 2) Reef type, 3) MPA status, and 4) Sites. If you can't recall this see the figure in the introduction above.

38. **Select** "***4***" ***factors***
39. **Click** OK.



40. **Double click** in the first cell below "***Factor***" (you will notice a drop down menu appears)
    a. **Select** "***Location***".
41. In the cell below "***Location***"
    a. **Select** "***Reef type***".
42. Continue down the column **selecting** "***MPA***" and "***Site***", in that order.

43. **Double click** on the cell next to "***Reef type***", under the column "***Nested in***"
    a. **Nest** "***Reef type***" in "***location***", as we discussed above.

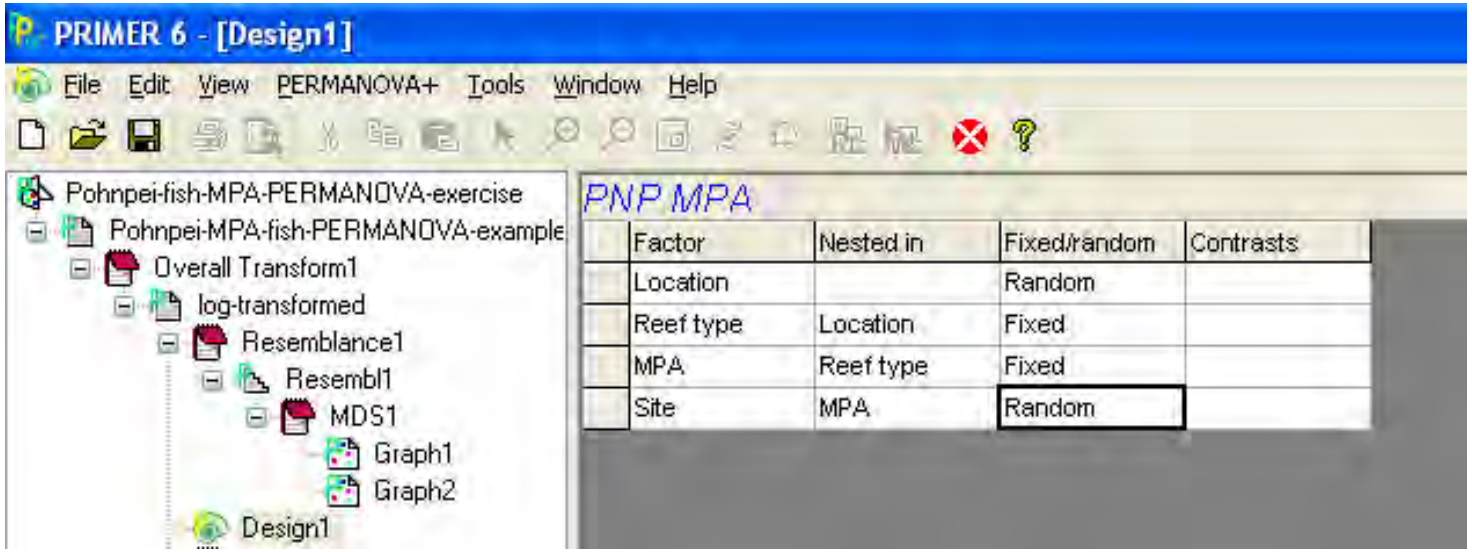


44. **Click** OK.
45. **Nest** "***MPA***" within "***Reef type***" (*as there are sites inside and outside of MPA's for each reef type*)
46. Finally, **nest** "***Site***" within "***MPA***" (*as there are two sites with 5 transects each inside each MPA zone*)

47. In the next column "Fixed/random" we need to
    a. Make sure the first box is set to "***Random***",
    b. The second and third to "***Fixed***"
    c. The fourth to "***Random***".

Our sampling design dictates whether or not a variable is fixed or random. For instance, "Location" could be any village in Pohnpei that decides to establish an MPA, so is set to random. However, "Reef type" and "MPA" status are well-defined categories that do not change and are not random by nature. Finally, "Site" or the exact placement of the sites in each MPA and reference site is also random.

48. Confirm.

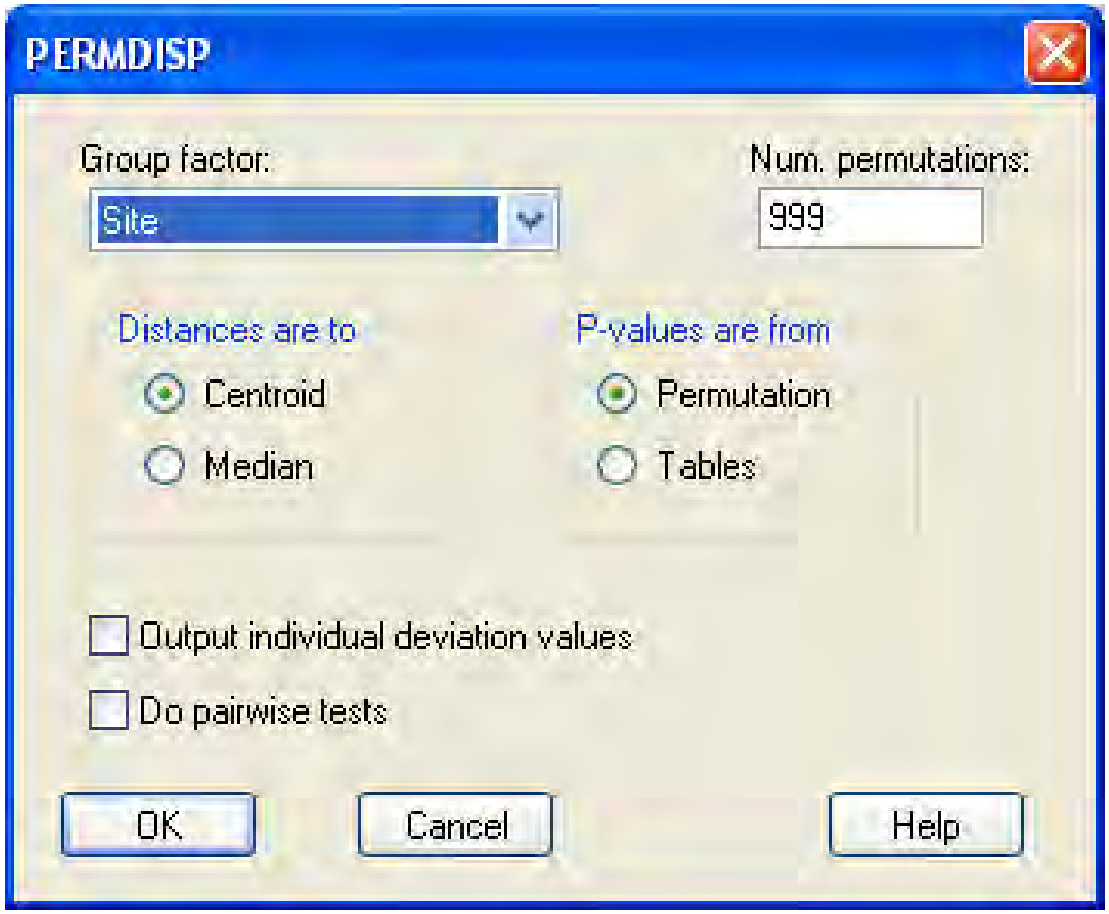


We are now ready to run our PERMANOVA on the dataset. However, just like any ANOVA test, we need to examine whether or not our variances are homogeneous. This will determine if we can continue with our PERMANOVA or we need to utilize a non-parametric test (i.e., a rank sum test procedure like an ANOSIM). Basically, can we use our actual numerical data, or do we need to use a derivative of the data, such as rank sums.

PRIMER has a function to understand the dispersion of the multivariate data. Dispersion can be thought of as statistical variance, or how different each replicate measure is to the next. For our example we wish to know if the replicate transects from each site all have similar levels of dispersion. If they do, we can move forward with our PERMANOVA, else, we'd probably choose to move on with the ANOSIM procedure, discussed above in Exercise 7.

<u>Remember we removed one outlier point so we need to calculate another similarity matrix from our log-transformed data.</u>

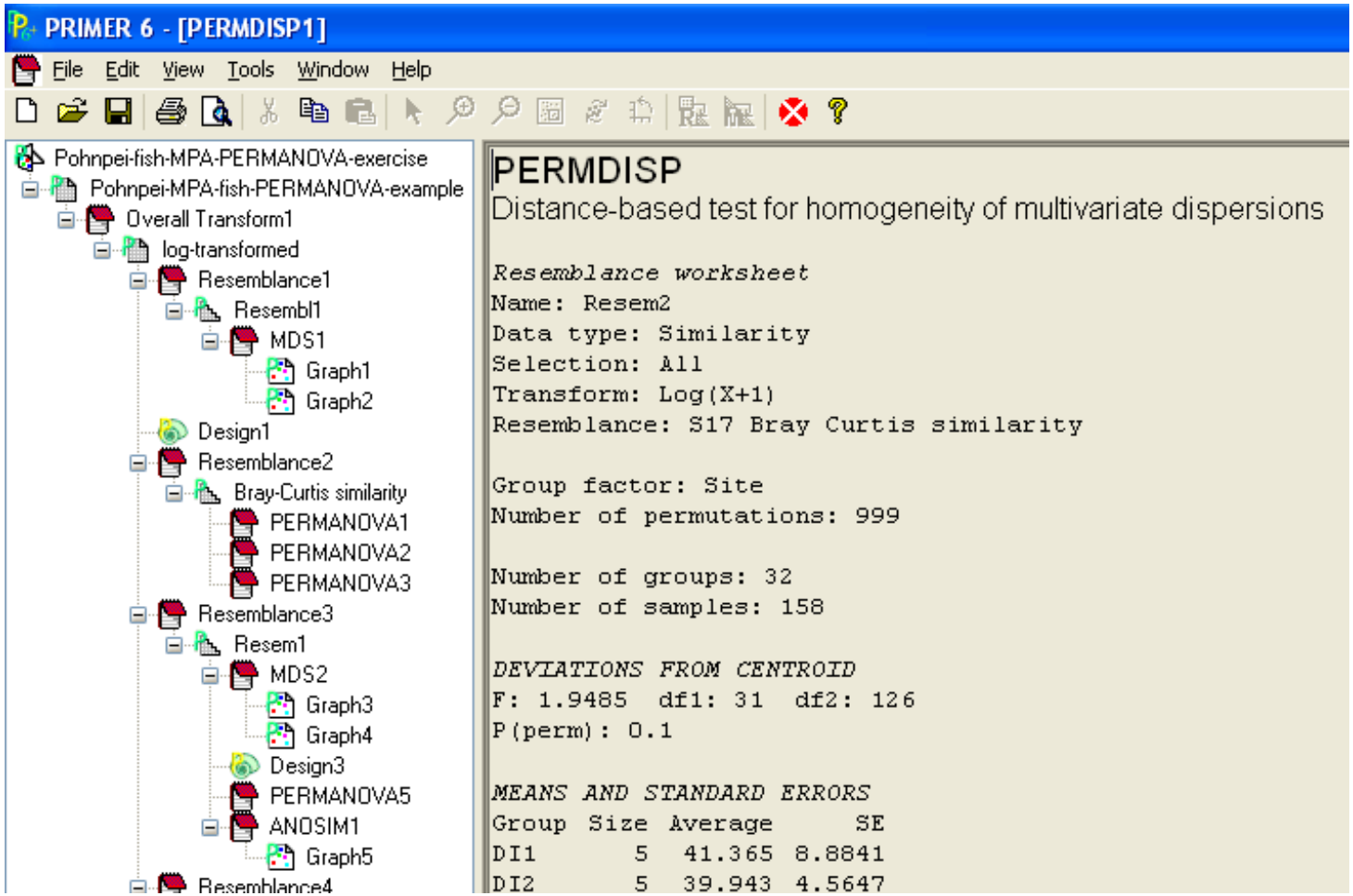
49. **Highlight** the "***log-transformed***" data sheet on the left.
50. **Go to** the "***Analyse***" menu
    a. **Scroll down** to "***Resemblance***"
    b. **Create** a ***Bray-Curtis similarity matrix***.
51. **Go to** the ***PERMANOVA***+ menu
52. **Scroll down** to ***PERMDISPERSE***. *The following dialog box should appear*



53. **Change** the group factor to "***Site***"

Meaning that we want to understand the variance at the site level, within which five replicate transects of data were collected. You can refer back to the diagram at the top if you don't understand why we are choosing "Site".

54. Click OK.



You should now get a PERMDISP results sheet that displays the homogeneity of multivariate dispersions. These results can be interpreted like and ANOVA F-statistic. The key results are located under the "Deviations from Centroid" header. Here you can see that our F-statistic is relatively low (1.9), and that we have 32 total sites, meaning there are 31 degrees of freedom for the test. The last item displays the P-value, P(perm) = 0.1, suggesting that no significant differences in multivariate dispersions exist between all of the sites. Below this you can find the average dispersion value for each site.

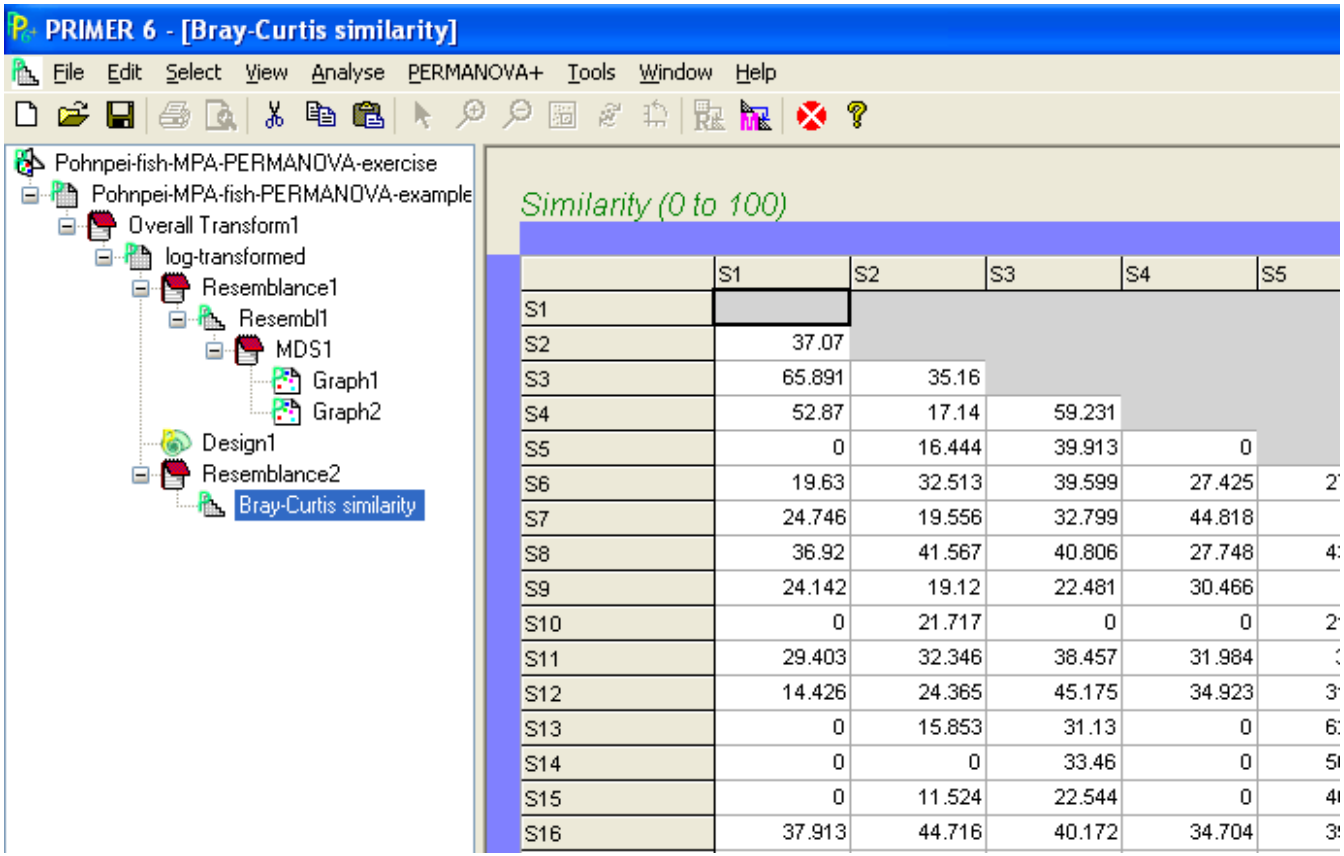
For our purposes, the non-significant value means that we can proceed as planned with our PERMANOVA, using the parametric dataset.

PERMANOVA Testing:

The input for a PERMANOVA test is a similarity matrix, such as our Bray-Curtis similarity matrix we created that describes how similar each individual transect is to one another.

55. On the left hand side of the screen **highlight** the "***log-transformed***" sheet.
56. Go to the "***Analyse***" menu
   a. Select "***Resemblance***" *make sure you are calculating a "Bray-Curtis" similarity matrix again*
57. Click OK.
58. Rename the resultant matrix "***Bray-Curtis similarity***".

See the previous exercise for a more formal definition of what this similarity matrix represents.

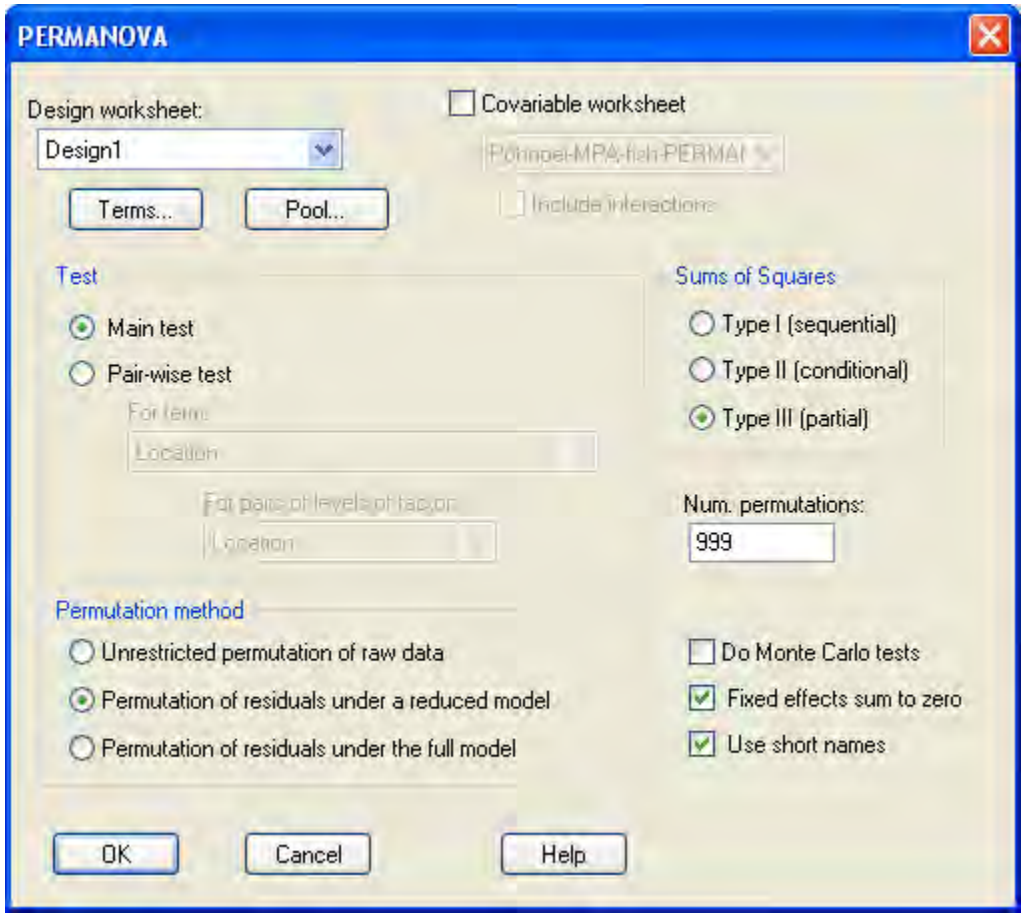


	S1	S2	S3	S4	S5
S1					
S2	37.07				
S3	65.891	35.16			
S4	52.87	17.14	59.231		
S5	0	16.444	39.913	0	
S6	19.63	32.513	39.599	27.425	2
S7	24.746	19.556	32.799	44.818	
S8	36.92	41.567	40.806	27.748	4
S9	24.142	19.12	22.481	30.466	
S10	0	21.717	0	0	2
S11	29.403	32.346	38.457	31.984	
S12	14.426	24.365	45.175	34.923	3
S13	0	15.853	31.13	0	6
S14	0	0	33.46	0	5
S15	0	11.524	22.544	0	4
S16	37.913	44.716	40.172	34.704	3

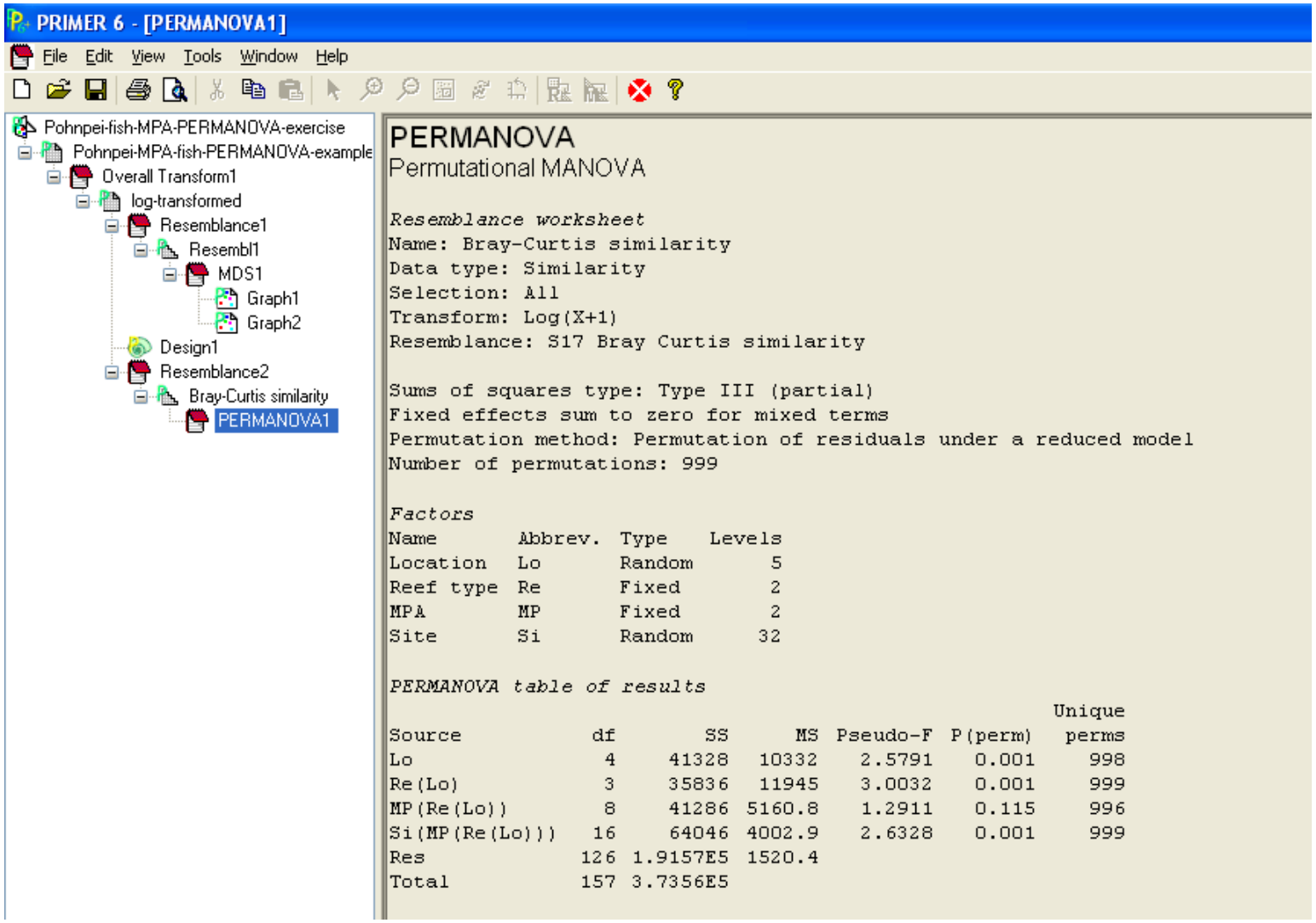
59. **Go to** the ***PERMANOVA+*** menu
    a. **Select** "***PERMANOVA***".
60. Under "***Design Worksheet:***" **select** "***Design1***"
61. Under "***Test***"
    a. **Select** "***Main test***".

You can use the default settings for the rest. The user guide has detailed explanations of each, we have selected the most common and general settings for now.

62. **Click** OK.



After a bit of computation, you should get a PERMANOVA results sheet.



The resultant data sheet starts with a summary of what you did to your dataset, and what type of factors you have, and how they were nested. Next, and most notable, you will find the PERMANOVA table of results. You can read this just like you would read an ANOVA table output. To better understand the calculations and logic refer to the user manual.

Here we see that "Location" was a significant predictor of the fish assemblages, suggesting significantly different assemblages exist within each village location, when looking at all transects together, regardless of reef type or MPA status. This is somewhat surprising considering our initial look at the MDS plot did not reveal easily interpretable differences. Nonetheless, the formal test of significance is our most thorough evidence.

Second, once location is accounted for, we also see that "Reef type" is a significant predictor of fish assemblages, and it actually had the highest F-statistic, suggesting its greater influence compared with "Location". This is not surprising either as our initial investigation of the MDS plot suggested this.

Third, we see that MPA status did not consistently predict any variance in fish biomass. Again, given what we found out in Exercise 3 and 4, where we noted that some MPA's were indeed successful and others not as much, this is expected, especially when looking at all of them together.

Finally, and notable, there was a significant amount of variance explained by "Site". This means that in the majority of instances sites within specific "Reef types", and within a specific "MPA status", can be quite different. ***Thus, it would be incorrect to lump the data from the two sites together to judge MPA status.*** *Rather, we might do better by considering each site independently.*

Below the table of results you can find descriptions of the numerical models that best fit our dataset. Last, the final "Estimates of components of variation" table shows us what the relative influence of each variable is, similar to estimates of individual ANOVA sum of square means. Individually, you can see the greatest components of variation exist at the "Site" and "Reef type" level, again supporting our initial MDS plot analysis. The user manual can help to understand these computational terms better, here we highlight the bottom-line findings and follow logical steps suggested by our sequential analysis.

So, what do we know?

There is a lot of variation among individual sites even if they are in the same "Location", "Reef Type", and "MPA" status. We might not desire to combine data from sites to judge a higher-order variable such as "MPA" status. However, the experimental design was set up to do this, recall "Site" was selected to be a random factor. So, we will proceed with our experimental design, but keep our knowledge gained in mind.

63. Go back to our "***Bray-Curtis similarity***" sheet.

We are ready to do a pairwise comparison to learn about the success of MPA status for each location and reef type, separately.

64. From the "***PERMANOVA***" menu
65. Select "***PERMANOVA***"

   a. **Change** our "***Test***" to "***Pair-wise***"

66. From the drop down menu below, **select** "***MPA(Reef type(Location))***"

67. Click OK.

68. Confirm the second PERMANOVA results sheet below.

```
PAIR-WISE TESTS

Term 'MP(Re(Lo))'

Within level 'D' of factor 'Location'
Within level 'Inner' of factor 'Reef type'
                        Unique
Groups       t  P(perm)   perms
Yes, No  1.293     0.35       3

Denominators
Groups   Denominator       Den.df
Yes, No  1*Si(MP(Re(Lo)))       2

Average Similarity between/within groups
        Yes      No
Yes  28.871
No   27.048  43.856

Within level 'K' of factor 'Location'
Within level 'Inner' of factor 'Reef type'
                          Unique
Groups         t  P(perm)   perms
Yes, No  0.73242        1       3
```

Here we can see the results of the pair-wise comparisons. The box above (i.e., the screenshot) highlights the first pair-wise comparison from inside of Village "D", on the "Inner Reefs", and we can see that a non-significant t-statistic and P-value emerged.

We can continue to scroll down and view each of the pair-wise results, however none are significant. We have a good idea as to why this is, and that is due to the "**Site**" level variation that exists. Let's confirm this.

69. Go back to your "***Bray-Curtis similarity***" sheet.

70. Go to the ***PERMANOVA*** menu

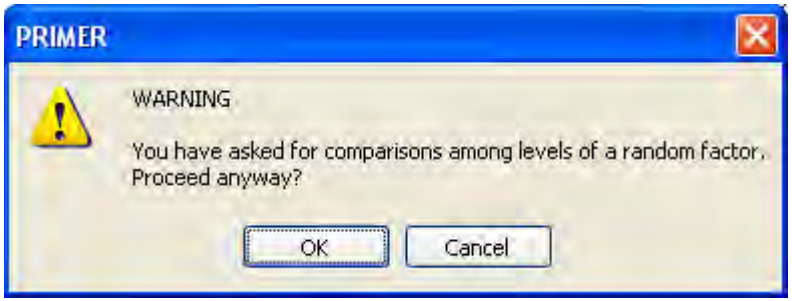
   a. Select "***PERMANOVA***".

71. Select pair-wise

   a. This time in the drop down box **select** "***Sites***".

72. Click OK.

***Note** : When the warning box appears you can click OK, we're well aware of our study design and we simply wish to view and understand the results so we can best move forward.*



73. Confirm the third PERMANOVA sheet.

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Bray-Curtis similarity
Data type: Similarity
Selection: All
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 999

Factors
Name       Abbrev.  Type    Levels
Location   Lo       Random       5
Reef type  Re       Fixed        2
MPA        MP       Fixed        2
Site       Si       Random      32

PAIR-WISE TESTS

Term 'Si(MP(Re(Lo)))'

Within level 'D' of factor 'Location'
Within level 'Inner' of factor 'Reef type'
Within level 'Yes' of factor 'MPA'
                          Unique
Groups         t  P(perm)   perms
DI1, DI2  1.5671    0.043     126

Denominators
Groups    Denominator  Den.df
DI1, DI2  1*Res             8

Average Similarity between/within groups
        DI1     DI2
DI1  32.372
DI2  23.973  37.613

Within level 'D' of factor 'Location'
Within level 'Inner' of factor 'Reef type'
Within level 'No' of factor 'MPA'
                          Unique
Groups         t  P(perm)   perms
```

A review of these site comparisons reveals that approximately 50% of pair-wise comparisons were significant. This confirms our thoughts that "Site" level variation is very high and needs to be accounted for, and probably is driving our non-significant findings of the differences between MPA status. While clearly we expected some site-level variation, we also expected that in some instances, MPA status might have a stronger influence on the fish biomass than reported.

While not the results we expected, it seems that a road for future analyses has been defined. In the future, we might want to remove the "nesting" of site within MPA status, and draw comparison among all sites from each corresponding reeftype, within each MPA locality. This is a logical step for Pohnpei's program to consider. Here, we begin to move in this direction in the final steps of our exercise.

Just a word of caution at this point. We can think of several reasons as to why our results came about, ranging from data-collector variance (i.e., different people collecting data from different locations), number and choice of indicator species, number, length, and width of transects, quality of the data collection, and most importantly, the length of time any particular site has been an established and enforced MPA. We have to be careful in using "absolute" terms when describing findings. Our discussion above describes the most probable causes for our findings.

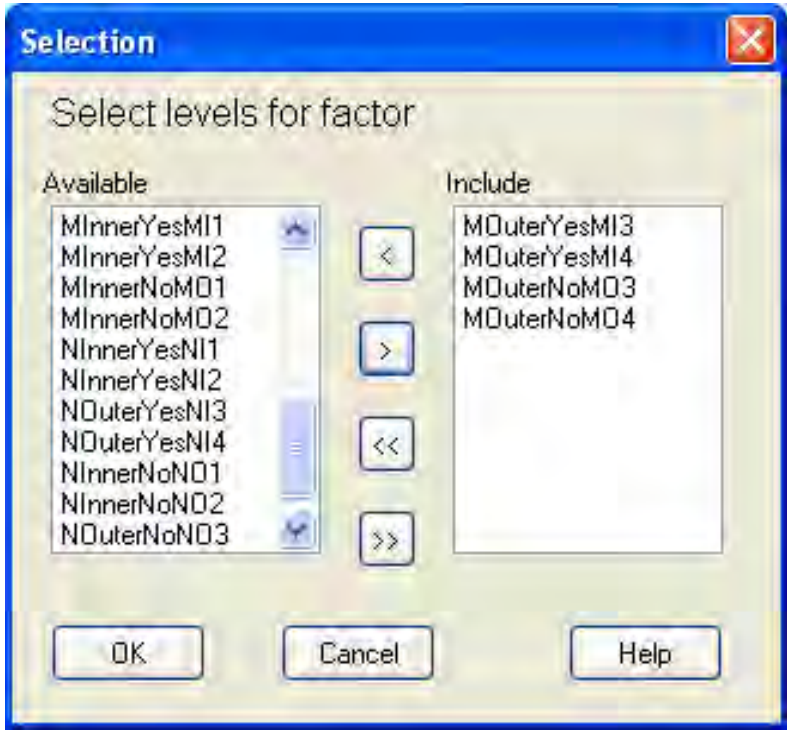
Graphical interpretations:

Our last exercise will be to look at and graph specific comparison between all sites for one of the MPA's. This was defined as the logical next step to take by our preliminary analyses. However, here we will only do this for one of the MPA's.

74. **Click** onto our "***log-transformed***" sheet.
75. **Click** on the "***Select***" menu and click "***Samples…***"
    a. **Check** "***Factor level***".
    b. In the drop down menu **highlight** "***Combined name***" and
    c. **Click** on the "***Levels***" box.

We will only consider village "M", and the "Outer" reefs in both MPA status.

76. In the "***Include***" dialog box **select** "***MOuterYesMI3***", "***MOuterYesMI4***", "***MOuterNoMO3***", "***MOuterNoMO4***".
77. Confirm with the image to the right.
78. **Click** OK in **all** dialog boxes.



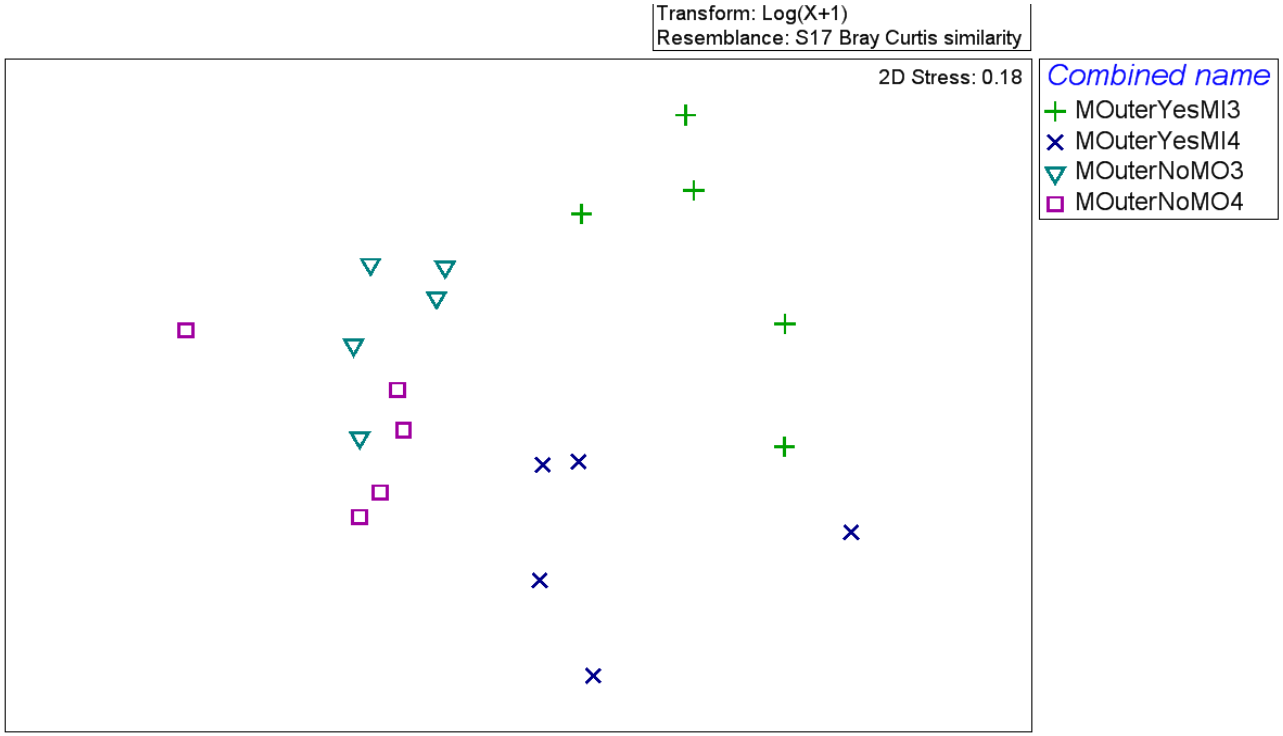
You should now have a subset of your desired sample transects.

79. Next, under "***Analyze***",
   a. Go to "***Resemblance***" and
   b. Create a ***Bray-Curtis similarity matrix***.

80. While keeping you matrix sheet active,
   a. Go to "***Analyze***" again and
   b. Select MDS plot.

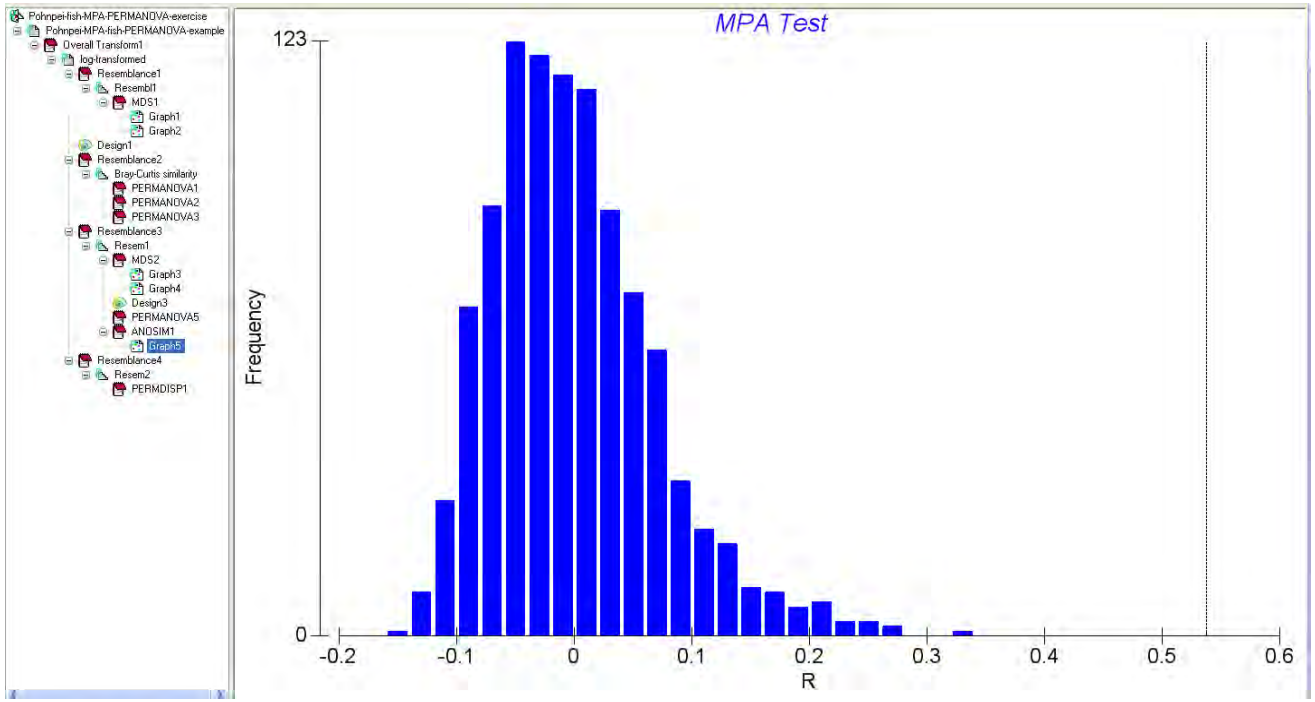
81. Click OK for the default settings.

82. From the graph page, **select** "***Data labels & symbols***"
   a. Uncheck the "***Plot***" box for labels.
   b. On the right, **select** "***Combined name***" from the dropdown menu
   c. Click OK
   d. Confirm your informative plot.



From this MDS plot it appears there are strong differences between individual sites, but also between inside and outside the MPA's. This is exactly the situation we thought was occurring from our initial analyses. However, we have to consider how these MDS plots are made before we wonder why non-significant findings were made in our PERMANOVA above where both sites were combined within each MPA status prior to examining for significance. MDS plots use non-parametric rank ordering of the inter-site differences. Meaning, rather than using the actual distances reported in the Bray-Curtis similarity matrix between any two sites, they simply rank the inter-site differences and capture the relative spread in two-dimensional space, described in ***Exercise 7****. Just as an exercise lets run an associated non-parametric test of significance now. This procedure is called an* ***ANOSIM,*** *short for analysis of similarities.*

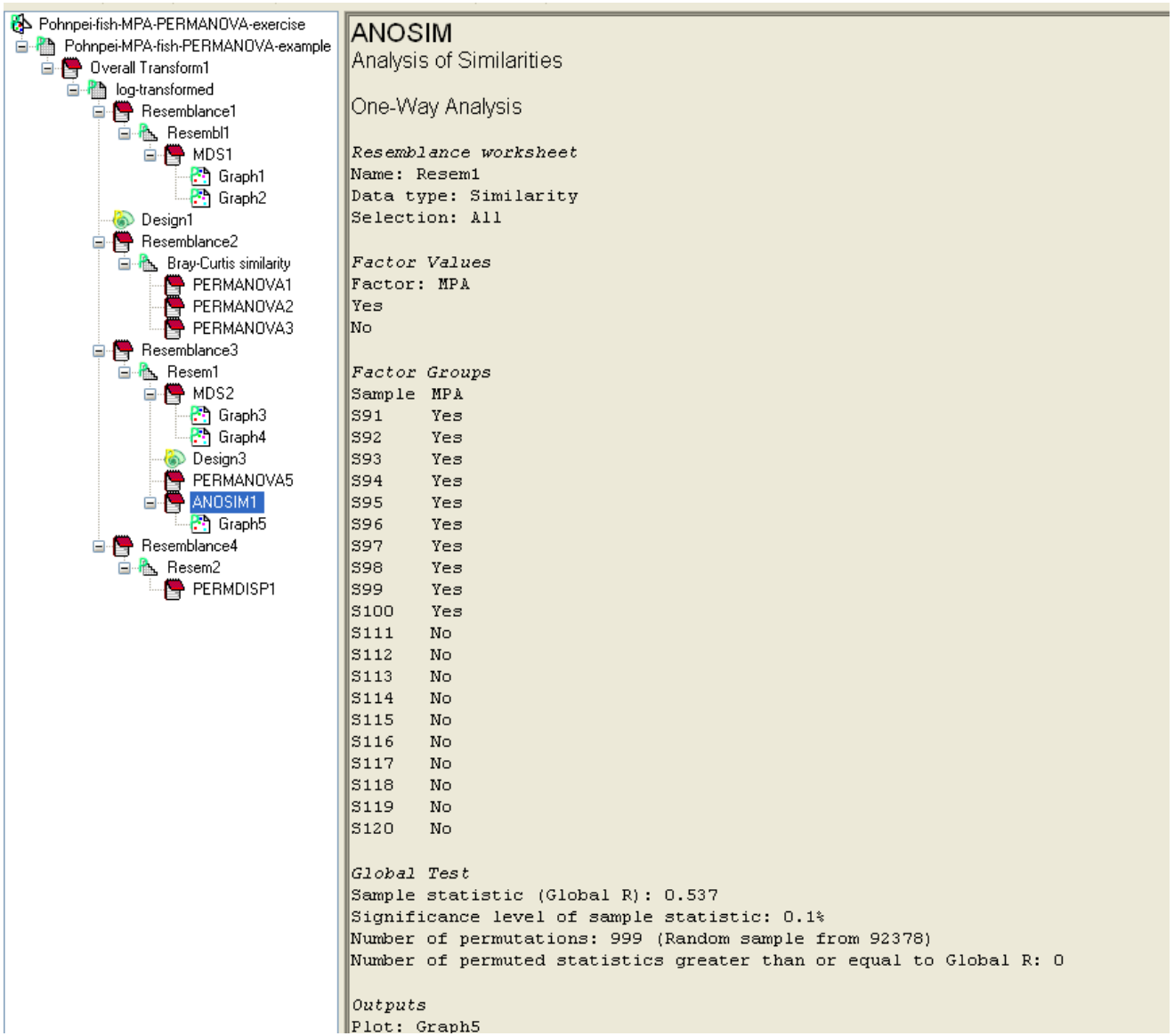
83. Highlight your "***Resem1***" similarity matrix associated with this plot.
84. Go to the "***Analyze***" menu and
   a. Scroll down to the ***ANOSIM***.
85. Select a "***one way***" design
   a. Set "***Factor A***" to "***MPA***". (*This will let us evaluate significant differences between all sites located within MPA's and all sites located outside of MPA's*)
86. Click OK
87. Confirm.



The first graph that appears shows the variation in the permutated R values that were calculated, typically a frequency distribution centered around 0 is desired to show that the procedure was successful, and that the calculated R-statistic is consistent.

88. Click on the ***ANOSIM*** spreadsheet on top of this graph page

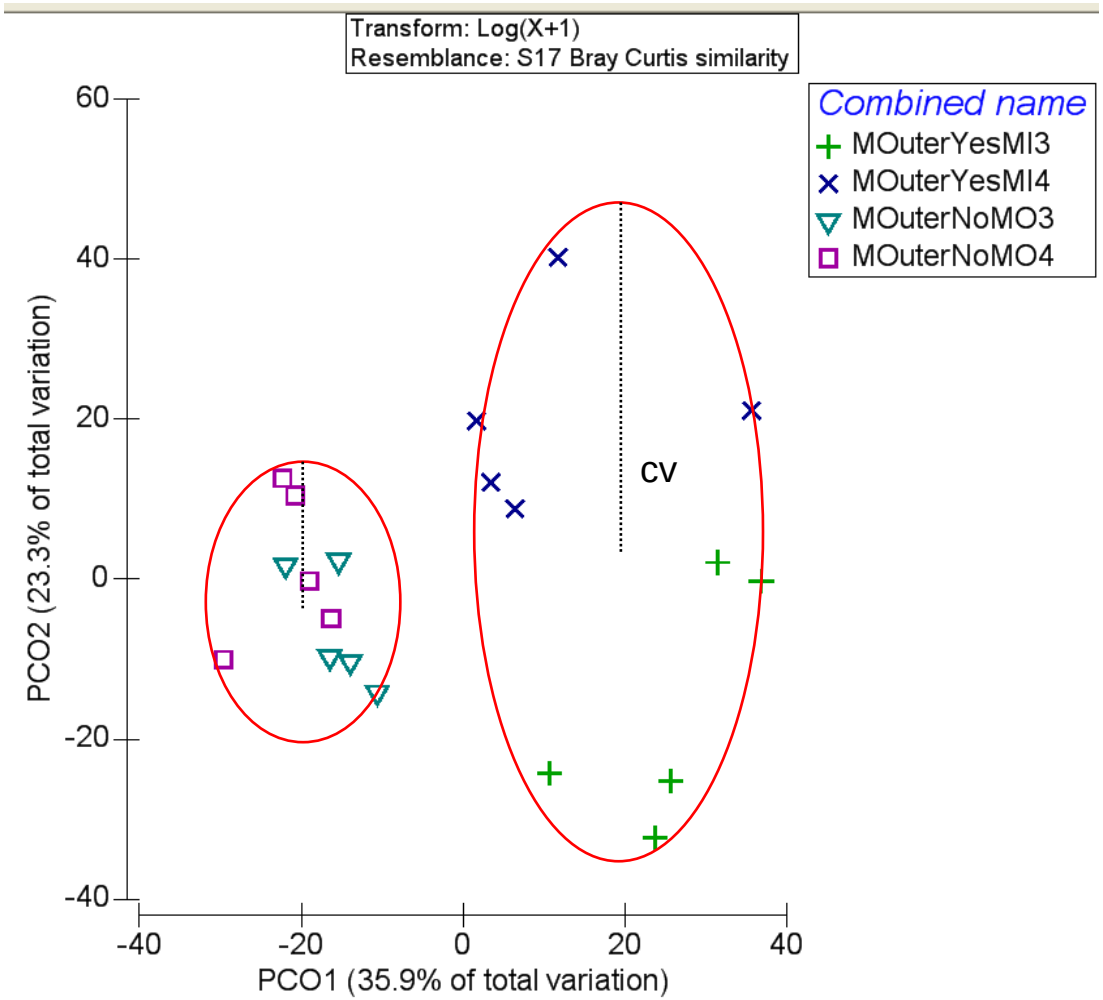
a. Scroll down to the results for the "***Global Test***".



You can see our R-statistic is 0.537, and P-value is 0.1% or 0.001. The guidance materials in the PRIMER book describes how to interpret significance using ANOSIM. Without getting into details provided there, the guidance suggests that any R-statistic above 0.5 can be considered statistically significant. Thus, the ANOSIM detects significant differences between MPA status that the PERMANOVA did not. We should again understand this may be due, in part, to the non-parametric ranking procedure.

Our last procedure here will be to prepare a PCO plot, rather than a MDS plot. PCO, or Principal Coordinate Ordination is a parametrical approach to produce informative plots such as MDS, using the actual values of the Bray-Curtis similarity matrix. PCO plots correspond to PERMANOVA test results.

89. **Highlight** the "***Resem1***" similarity matrix used with our ***ANOSIM***.
90. **Click** on the ***PERMANOVA*** menu
91. **Scroll down** to ***PCO***.
92. **Keep** all default settings
93. **Click** OK.
94. From the graph page, **select** "***Data labels & symbols***".
    a. **Uncheck** the "***Plot***" box for labels.
    b. On the right, **select** "***Combined name***" from the dropdown menu.
95. **Click** OK
96. Confirm your informative plot, noting that red circles and black dashed lines were only drawn in here for explanatory purposes described below.



We can see clear similarities between our PCO plot and our MDS plot. To better understand why we didn't get significant findings in our PERMANOVA you can envision the two data clouds circled above. These represent the two datasets we wish to detect significant differences in. The average radius of these circles represents the "component of variation" or basically the variance in the multivariate data. This is the dashed black line above with a "CV" next to it. These can be interpreted like standard deviation bars on our graphs. If the black dash line is longer that the mean distance between the two center points of our circle, the PERMANOVA will most likely show a non-significant result. Also different from the MDS plot, you can see numerical values on the X and Y axes.

The last clear message we can learn through our visualization of the PCO plot is that it seems the MPA status is, in fact, having an impact on the fish assemblages here. However, you can see the high inter-site variation between the two sites in the MPA boundary: 1) "MOuterYesMI3", the green plus signs, and 2) "MOuterYesMI4", the blue X. It appears both sites, individually, would be significantly different from the reference sites, but when combining the data from these sites to test for MPA effectiveness, too much variation is introduced.

Clearly our summary provides a wealth of information to inform the monitoring and management programs of Pohnpei. Some clear suggestions from exercises 3, 4, and 8 are that the MPA's have a mixed success, and trends vary within each village. Even at sites where current improvements are noted, the results are not yet significant in all cases. ***This may be due to the confidence in our monitoring data, the amount of time since the MPA was established, or the level of compliance with the no-take fishing policy.*** *For the monitoring program these results suggest potential changes to their sampling design and/or methodologies. It seems very important to maintain the same data collector when conducting fish surveys. Similar, there may be a desire to expand data collection efforts to include all food fish, rather than the select indicator species. Finally, reducing the number of replicate stations in each MPA main represent one means of saving time and funding to accomplish the other deficiencies.*

A last note is that there are several ways in which these data could have been analyzed using PERMANOVA. We might want to consider further tests at the individual site level, rather than grouping the sites to determine whether or not MPA are successful. Clearly this would be a positive next step, but entails somewhat of a revision of the ecological sampling plan. These are all terrific points for monitoring programs to discuss with each other and with scientific advisors.

End of Exercise 8